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A STUDY OF THE BLOOD-UREA CLEARANCES WITH RELATION TO DIURESIS IN NORMAL AND NEPHRITIC ANIMALS*

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THE purpose of this investigation is to show the close correlation which exists between the diuresis curve in the fasting mammal and the curve of blood-urea clearance. Certain important papers are briefly reviewed below; these serve as a basis of comparison, as well as to explain the terminology used.

Investigations by Möller, McIntosh, and van Slyke[1] confirm those of Marshall and Davis,[2] Pepper and Austin,[3] Addis and Watanabe,[4] that when the volume of urine is fairly large the rate of urea excretion is directly proportional to the blood-urea content. They call attention to the fact that Austin, Stillman, and van Slyke[5] demonstrated in three normal subjects that the rate of excretion holds only when the urine volume is above a certain limit (about 2 c.c. per minute in adults) which they called the "augmentation limit." When the volume of urine became less than the so-called augmentation limits of the subjects studied, the urea output decreased in proportion to the square root of the volume. This conception is confirmed by Möller, McIntosh, and van Slyke on seven other human subjects. Austin, Stillman, and van Slyke[5] considered that a further increase in the volume of urine would not result in a further increase in the hourly output of urea.

Möller, McIntosh, and van Slyke[1] stated, furthermore, that in adults, when the excretion of urine is proceeding at the rate of 2 c.c. or more per minute, a certain volume of blood will be freed of urea each minute. They found that in men with normal kidneys this volume of blood was approxi-

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mately 75 c.c. per minute, and called this figure the maximum blood-urea clearance, designated C_m. They found also that in men this maximum clearance is practically a constant figure for all urine volumes equal to or above the so-called augmentation limit (of about 2 c.c. per minute). Under conditions of diuresis, however, in which the urine excretion fell appreciably below 2 c.c. per minute, the amount of blood which was cleared of urea diminished, and another basis of calculation was necessary for such instances. It was found that as the volume of urine diminished more and more, the diminution of the number of cubic centimeters of blood cleared of urea per minute was not exactly proportional to the volume of urine, but was more nearly proportional to the square root of the volume when this volume was below the augmentation limit of 2 c.c. per minute. For such conditions, the authors coined the term "standard clearance," which they defined as *the efficiency with which the kidneys excrete urea when the volume of urine is at the average normal level of 1 c.c. per minute* (or 1,440 c.c. per twenty-four hours), designated as C_s. The formula for standard clearance, as derived by these authors is:

$$C_s = U/B \sqrt{v} \quad (1)$$

For maximum clearance, the formula is:

$$C_m = UV/B \quad (2)$$

U designates the milligrams of urine urea per 100 c.c., *B* the milligrams of blood urea per 100 c.c., and *V* the volume of urine per minute.

As already stated, the second of these formulae was used for adults only when the urine volume was 2 c.c. or more per minute. For adaptation of the formulae to extremely heavy or extremely thin adults, or to children, the authors recommended that the value for *V* (urine volume) be multiplied by the formula introduced by Addis:

$$1.73/\text{Sq. Meters Surf. Area} \quad (3)$$

A complete discussion of the use of this formula is given by McIntosh, Möller, and van Slyke.[6] These authors are in accord with MacKay and MacKay[7] that loss of renal function may exceed 60 per cent before the blood-urea content rises above the highest level observed in normal subjects, and they state that unless the excretion rate is also considered, the blood urea may fail to reveal diminishing renal ability until the latter has become advanced.

Eaton M. MacKay[8] observed that urine volume was not the only important factor affecting urea excretion. He investigated the diurnal variation of the standard blood-urea clearance and found this variation to be considerable in each individual under normal conditions, and that a further variation is brought about by exercise. His experiments indicate that there is less variability in a series of observations made on an individual at the same hour on different days than in a series of observations made on the same day.

EXPERIMENTAL DATA

Before undertaking this study on dogs, we conducted 28 experiments in diuresis on a fasting subject with normal renal function under varying conditions of activity. Some of these experiments were done with the subject

in bed, others while doing sedentary work, and still others during the performance of mild exercise. Similar diuresis tests were conducted on four other healthy subjects and four with nephritis, determined by at least two criteria in each case. The experiments done on fasting human subjects differed from those done on dogs in that the human beings drank the two 500 c.c. portions of water, and urine specimens were passed voluntarily, a procedure which is satisfactory when there is no pathologic condition causing urinary retention. In all of the 32 tests on nonnephritic human subjects the diuresis curves were typical of those obtained in normal dogs, there being a high peak of excretion within two hours, with a subsequent abrupt decrease of the excretion rate to a point near the fasting level within five hours. The curves in nephritic human subjects, however, lacked the normal high peak

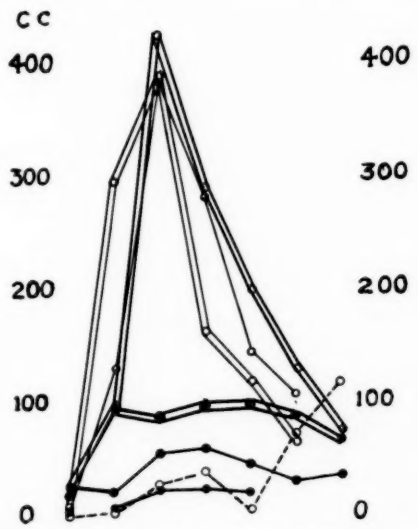

Fig. 1.—Curves of water diuresis, hourly specimens, in normal and nephritic animals. The higher, open-circle curves are of nonnephritic animals. The lower, black-dot circles are of nephritic animals. The double-line curve indicated results following administration of 1,000 c.c. of 5 per cent urea, the single-line curve following 1,000 c.c. of water, and the dotted-line curve following 1,000 c.c. of 5 per cent urea and 1 c.c. of surgical pituitrin.

within the first two hours and failed to reach an equilibrium within five hours. Such tests are distinctly helpful, but are not intended to replace studies of the blood chemistry.

Our observations of standard and maximum blood-urea clearances in dogs were made both before and after the induction of acute nephritis by means of uranium acetate, 2.5 mg. per kilogram subcutaneously. Fig. 1 shows the results in graphic form. Fig. 2 shows graphically the urine volumes noted in the tables. The bases of computation of these blood-urea clearances are given in Tables I to VII. (A rapid survey may be made of these tables by reading the extreme left- and right-hand columns together.)

It is noted that when large doses of urea are given to dogs with normal renal function the absolute amounts of urea excreted and the volume of urine are very greatly increased. This mechanism may serve as a rapid means of dehydration. The number of cubic centimeters of dog blood cleared

of urea per minute is definitely decreased in most instances, both in the normal and in the nephritic animal; but in our hands the decrease was negligible if pituitrin was given at the same time as the urea.

The designation *maximum blood-urea clearance* which has been applied to man is a misnomer for the dog. McIntosh, Möller, and van Slyke conclude that about 75 c.c. is the average quantity of blood which can be cleared of urea per minute in healthy men when the urinary output is 2 c.c. or more per minute, and it is inferred that this, generally speaking, is the maximum

TABLE I

HOUR OF EXPERIMENT	URINE VOLUME, C.C. PER HOUR	URINE VOLUME, C.C. PER MIN.	SQ. RT. OF VOL. PER MIN.	URINE UREA, GM. PER 100 C.C.	BLOOD UREA, GM. PER 100 C.C.	URINE UREA, GM. PER HOUR	BLOOD-UREA CLEARANCE, C.C. PER MIN.
control	6.5	0.10	0.33	4.77	0.024	0.310	65 (standard)
1	7.0	0.11	0.33	3.51	0.021	0.245	55 (standard)
2	36.0	0.60	0.78	2.58	0.027	0.928	74 (standard)
3	44.0	0.73	0.85	2.34	0.021	1.029	94 (standard)
4	30.0	0.50	0.71	2.28	0.024	0.684	67 (standard)
5	78.0	1.30	1.14	1.14	0.060(?)	0.889	—
6	122.0	2.03	1.42	0.60	0.033	0.732	26 (standard)

Dog No. 5, normal, before induction of nephritis. The table shows the basis of computing the hourly blood-urea clearances following two doses of 500 c.c. each of water only, given by stomach tube, and 1 c.c. of surgical pituitrin given subcutaneously.

TABLE II

HOUR OF EXPERIMENT	URINE VOLUME, C.C. PER HOUR	URINE VOLUME, C.C. PER MIN.	URINE UREA, GM. PER 100 C.C.	BLOOD UREA, GM. PER 100 C.C.	URINE UREA, GM. PER HOUR	BLOOD-UREA CLEARANCE, C.C. PER MIN.
control	33.5	0.56	3.84	0.027	1.286	10.6 (standard)
1	102.0	1.70	2.73	0.207	2.784	22.4 (maximum)
2	427.0	7.11	2.82	0.240	12.041	83.0 (maximum)
3	295.0	4.91	3.15	0.213	9.292	72.0 (maximum)
4	206.0	3.43	2.76	0.147	5.685	63.8 (maximum)
5	136.0	2.26	3.84	0.132	5.222	65.7 (maximum)
6	80.0	1.33	5.34	0.120	4.272	57.8 (maximum)

Dog. No. 3, normal, before induction of nephritis. The table shows the basis of computing the hourly blood-urea clearances following two doses of 500 c.c. each of 5 per cent urea solution given by stomach tube, and 1 c.c. of surgical pituitrin given subcutaneously.

TABLE III

HOUR OF EXPERIMENT	URINE VOLUME, C.C. PER HOUR	URINE VOLUME, C.C. PER MIN.	URINE UREA, GM. PER 100 C.C.	BLOOD UREA, GM. PER 100 C.C.	URINE UREA, GM. PER HOUR	BLOOD-UREA CLEARANCE, C.C. PER MIN.
control	14	0.23	5.70	0.039	0.798	70.5 (standard)
1	302	5.03	1.80	0.330	5.436	28.8 (maximum)
2	395	6.58	2.07	0.249	8.176	55.0 (maximum)
3	172	2.86	3.30	0.231	5.211	40.0 (maximum)
4	123	2.05	4.59	0.204	5.545	45.0 (maximum)
5	74	1.23	5.40	not taken	3.996	——

Dog. No. 3, normal, before induction of nephritis. The table shows the basis of computing the hourly blood-urea clearances following two doses of 500 c.c. each of a 5 per cent solution of urea given by stomach tube. It is to be noted that under stress of high blood urea, the kidney clears less blood per minute.

amount of blood which can be cleared of urea during the most forced diuresis. Certainly this does not apply to dogs, and it is reasonable to expect a difference in view of the variation in surface area. This difference is further emphasized after the administration of large amounts of urea by mouth. Doubtless the results of diuresis tests are also affected by the plane of protein metabolism existing at the time of the test.

It seems very probable that the secretion of the pars intermedia (the active principle of pituitary extract) is an important factor in determining the output of urea, and should take its place as such along with other factors which influence the quantity of blood cleared of urea per minute; namely, magnitude of urine volume, diurnal variation, exercise, and other conditions which alter the flow of blood through the kidneys. It is emphasized that

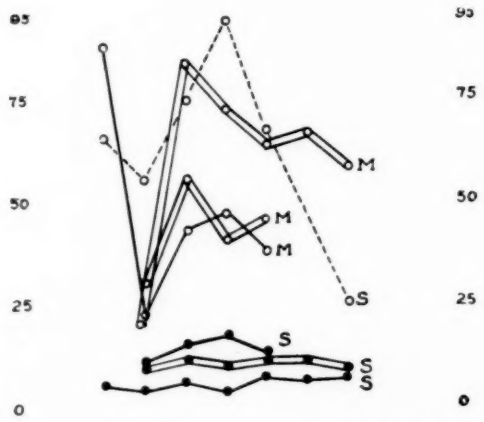

Fig. 2.—Curves of blood-urea clearances. Those marked *M* are based on the formula for maximum clearance; those marked *S* are based on the formula for standard clearance. The dosage corresponds to the line designations in Fig. 1. All charts are constructed from the tables.

while pituitary extract is known to alter the blood flow through the kidneys in a classical manner, and at the same time is noted to diminish urine volume (Tables II and VI), the amount of blood cleared of urea per minute in these experiments does not share in this decrease but may actually be increased. Further work is needed in connection with these facts.

TABLE IV

HOUR OF EXPERIMENT	URINE VOLUME, C.C. PER HOUR	URINE VOLUME, C.C. PER MIN.	URINE UREA, GM. PER 100 C.C.	BLOOD UREA, GM. PER 100 C.C.	URINE UREA, GM. PER HOUR	BLOOD-UREA CLEARANCE, C.C. PER MIN.
control	9	0.15	6.12	0.027	0.550	87.0 (standard)
1	137	2.28	1.71	0.192	2.330	20.4 (standard)
2	393	6.55	1.62	0.246	6.366	42.8 (standard)
3	287	4.78	2.31	0.234	6.629	47.0 (standard)
4	150	2.50	3.0	0.198	4.500	38.0 (standard)
5	115	1.91	3.48	not taken	4.000	——

Dog. No. 5, normal, prior to induction of nephritis. The animal was catheterized, and one 500 c.c. dose of water was given immediately, a second 500 c.c. dose being given thirty minutes later. The table shows the basis of arriving at the standard urea clearances.

The curves of blood-urea clearance in the normal and nephritic animal and the type of curve of urine volume (with active diuresis), as given in Figs. 1 and 2, should be compared. These curves begin with the fasting state, and are carried through the period of copious diuresis, hourly specimens being taken until equilibrium is practically restored.

TABLE V

HOUR OF EXPERIMENT	URINE VOLUME, C.C. PER HOUR	URINE VOLUME, C.C. PER MIN.	SQ. RT. OF VOL. PER MIN.	URINE UREA, GM. PER 100 C.C.	BLOOD UREA, GM. PER 100 C.C.	URINE UREA, GM. PER HOUR	BLOOD-UREA CLEARANCE, C.C. PER MIN.
control	none obt.	——	——	——	0.069	——	——
1	11.0	0.183	0.42	1.80	0.069	0.198	10.90 (standard)
2	28.0	0.466	0.68	1.35	0.060	0.378	15.07 (standard)
3	30.0	0.500	0.71	1.35	0.054	0.405	17.70 (standard)
8	24.5	0.408	0.64	1.11	0.051	0.271	13.80 (standard)

Dog. No. 5, nephritic, five days after the subcutaneous injection of uranium acetate, 2.5 mg. per kilogram. The table shows the basis of computing the hourly standard urea clearances following two doses of 500 c.c. each of water given by stomach tube. The blood-urea clearance has fallen from a prenephritic level of 47 (for the third hour) to 17.7 c.c.

TABLE VI

HOUR OF EXPERIMENT	URINE VOLUME, C.C. PER HOUR	URINE VOLUME, C.C. PER MIN.	URINE UREA, GM. PER 100 C.C.	BLOOD UREA, GM. PER 100 C.C.	URINE UREA, GM. PER HOUR	BLOOD-UREA CLEARANCE, C.C. PER MIN.
control	25	0.41	1.35	0.108	0.337	——
1	100	1.66	2.04	0.339	2.040	9.9 (maximum)
2	95	1.58	2.43	0.321	2.308	11.9 (maximum)
3	105	1.75	1.86	0.303	1.953	10.7 (maximum)
4	105	1.75	1.86	0.282	1.953	11.1 (maximum)
5	95	1.58	1.86	0.273	1.767	10.7 (maximum)
6	72	1.20	2.19	0.264	1.576	9.95 (maximum)

Dog. No. 5, nephritic, seven days after the subcutaneous injection of uranium acetate, 2.5 mg. per kilogram. The table shows the basis of computing the hourly (so-called maximum) blood-urea clearances following two doses of 500 c.c. each of a 5 per cent solution of urea given by stomach tube. Of the second dose, 440 c.c. was vomited, but an equal amount of water was promptly given by stomach tube.

TABLE VII

HOUR OF EXPERIMENT	URINE VOLUME, C.C. PER HOUR	URINE VOLUME, C.C. PER MIN.	SQ. RT. OF VOL. PER MIN.	URINE UREA, GM. PER 100 C.C.	BLOOD UREA, GM. PER 100 C.C.	URINE UREA, GM. PER HOUR	BLOOD-UREA CLEARANCE, C.C. PER MIN.
control	33.0	0.55	0.74	1.05	0.144	0.346	5.3 (standard)
1	26.5	0.44	0.66	0.93	0.126	0.247	4.8 (standard)
2	80.0	1.33	1.15	0.54	0.114	0.432	5.4 (standard)
3	65.5	1.09	1.05	0.48	0.108	0.314	4.6 (standard)
4	50.0	0.83	0.91	0.66	0.099	0.330	6.0 (standard)
5	38.0	0.63	0.79	0.87	0.126	0.330	5.4 (standard)
6	40.5	0.67	0.82	0.90	0.120	0.364	6.1 (standard)

Dog No. 3, twelve days after the subcutaneous injection of uranium acetate, 2.5 mg. per kilogram. The table shows the basis of computing the hourly standard urea clearances following two doses of 500 c.c. each of water given by stomach tube, one dose immediately after the control hour, the other dose thirty minutes later.

PRACTICAL APPLICATION OF THE TEST

In this connection one point in particular should be emphasized; namely, that the majority of patients presenting themselves for diagnosis and treatment should have some evaluation of the renal status when it can be done so quickly and simply. The patient should be requested to come to the office after a twelve-hour fast, bringing a specimen of the night urine and one taken an hour after the completion of the night specimen. On arriving, he can be seated to await his turn. When called, he should be requested to empty the bladder, this specimen being discarded. He should then be given 500 c.c. of water to drink, and an additional 500 c.c. thirty minutes later. Beginning from the time of the first dose of water, urine specimens should

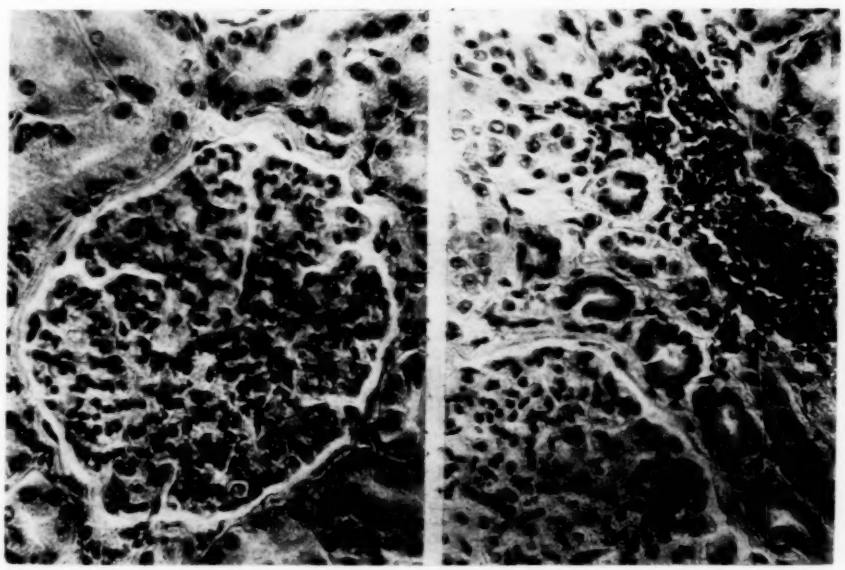

Fig. 3.—High-power views of a kidney from dog No. 3, nineteen days after the injection of uranium acetate subcutaneously, 2.5 mg. per kg.

be taken hourly for not more than six hours. The specific gravity and albumin content of the night specimen should be determined, and the volume and specific gravity of the remaining specimens.

A very satisfactory normal type of curve is one which gives a high volume peak in the second hour and an abrupt falling off of volume in the third, fourth, and fifth hours. The specific gravity is very low in the second hour, but gradually rises and approaches that of the night specimen by the fifth or sixth hour. The difference between the specific gravity of the night specimen or the sixth-hour specimen and that of the second-hour specimen will give a good estimate of the ability of the kidneys to concentrate urea, and this finding will be borne out by a subsequent determination of urea concentration if it seems necessary. If there is no good reason to suspect kidney damage, further tests are not indicated.

If the curve obtained is within the average, the following conditions will also be found, with but exceedingly rare exceptions:

1. The test of phenolsulphonephthalein excretion will be satisfactory.
2. The output of urea will be normal.
3. There will be no fixation of specific gravity.
4. The blood-urea clearance will be normal.
5. There will be no fixation of blood-urea nitrogen at a high level, and the blood-nonprotein nitrogen will be within normal limits.
6. The functional capacity of the circulation at rest will be adequate for good renal function.

If edema is present, a blood chloride determination is imperative. If this gives figures near normal, a sodium chloride tolerance test should be

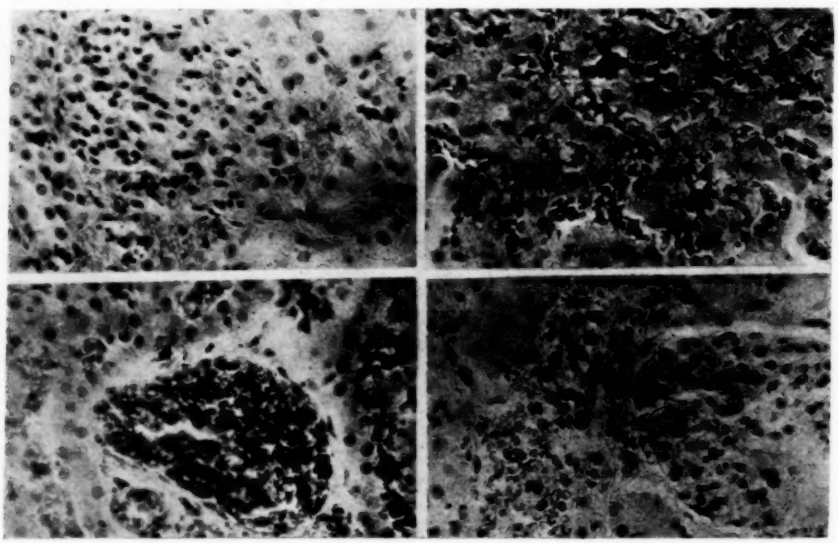

Fig. 4.—High-power views of a kidney from dog No. 5 seven days after the injection of uranium acetate subcutaneously, 2.5 mg. per kg.

done, with parallel determinations of chloride on the blood and urine. If the chloride elimination is normal and chloride feeding does not cause untoward symptoms, the amount of this important element in the diet should not be limited, or derangement of the acid-base equilibrium may result. If urea is tolerated well and eliminated normally, the diet should contain a fairly liberal amount of protein. In cases of nephritis with edema in which the patients tolerate urea well, Hugh McLean gives 15 gm. of urea by mouth daily for several days, alternating with a similar period in which no urea is given. The dehydrating effect of the urea reduces the edema in some of these cases just as administration of urea produces dehydration in normal dogs.

If focal infection is known to have existed for a long time or if there is a high diastolic pressure, arteriosclerosis, headache, mental inertia, and spots

before the eyes—a syndrome suggesting renal injury—a further study of the renal efficiency is certainly indicated. This should include: (1) some evaluation of the acid-base balance such as determination at least of the carbon-dioxide combining power of the plasma and the sodium-chloride content of the plasma or of the whole blood, (2) an estimation of the fasting blood urea, and (3) a study of the albumin and globulin fractions of the blood. The results of these tests, added to those of the diuresis curves and phenolsulphonephthalein excretion, should either give an insight leading to a definite diagnosis or indicate the need for other more specialized renal tests. If the carbon-dioxide combining power of the plasma is very low (35 or less), the patient should have glucose daily. When this is not tolerated by mouth (because of vomiting), it may be given intravenously, with insulin if the sugar tolerance is impaired. As pointed out by Peters, Wakeman, Eisenman, and Lee,[9] the glucose may save tissue protein from destruction and also diminish the ketosis.

Hospitals routinely force fluids on patients with nitrogen retention. A nephritic patient who receives three or more liters of fluid daily should have a determination of the corpuscle volume index to learn whether there is plethora or blood concentration, as an indication for decrease or increase of the fluid intake. Peters and his coworkers[9] found that hypochloremia and a low serum base are usually attended by anhydremia and general dehydration, and pointed out that if the blood chlorides are low, the forced water diuresis (which is part of the therapy against toxemia and anhydremia) may further deplete the chlorides to a very harmful extent unless the patient is given sodium chloride in the diet to the extent of about seven to ten grams daily with a fluid intake of 2,000 to 3,000 c.c.

These same authors[9] report sixteen determinations of serum albumin and globulin in twelve healthy subjects which averaged 5.19 per cent for albumin and 2.1 per cent for globulin—7.29 per cent total protein. Rowe[10] reports analyses in 22 cases with variations of serum albumin between 4.6 and 6.7 per cent, globulin between 1.2 and 2.3 per cent, total proteins between 6.5 and 8.2 per cent, with averages for serum albumin of 5.6 per cent, serum globulin 1.9 per cent, and total protein 7.5 per cent. This author used the method of Robertson.[11] In his review he reports definite increases in serum globulin in syphilis, and states that in pneumonia it is increased more in relation to the total protein than in syphilis, while the total protein is reduced. In many *chronic* septic conditions, he continues, in mild infections, and in typhoid fever, the total protein is not decreased as it is in pneumonia, while *globulin* seems definitely increased in all infections, except in acute tonsillitis, typhoid, and certain mild infections such as chronic bronchitis.

Howe[12] reviewed the physiology of the plasma proteins, and pointed out the conditions which may cause variation, such as species, age, water balance, activity, disease, etc., stating that under ordinary conditions the composition of the plasma of an individual is relatively constant. He considers that isolated determinations are of little value, and recommends a series

of such analyses following the course of the disease. With more general use of this test, it may become increasingly helpful in the classification of nephrities.

SUMMARY

Data are presented correlating the water-diuresis curve with the ability of the kidneys to clear the blood of urea in normal and nephritic animals.

A simple diuresis test may be used in patients of limited means to indicate the results which may be expected from certain more extensive, expensive, and time-consuming renal tests.

This research has been conducted under the James W. Packard Research Endowment Fund. The blood-chemical determinations were carried out by Miss Harriet Hippard, of the Department of Biochemistry, to whom thanks are extended. We desire also to express appreciation to Dr. John Tucker, of the Department of Medicine, for criticism of the manuscript.

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THE INCIDENCE OF LIPOIDS IN URINE*

Being a Report of the Micropolariscopic Examination of 1470 Specimens

By Arthur T. Brice, Jr., B.A., Florence, S. C.

THE researches of von Noorden, Munk, Genck, and Miloslavich[1] indicate the fact that in normal urine no anisotropic substances of lipoidal composition can be found. Miloslavich further emphasizes the experimental fact that during the course of an artificial hypercholesterolemia, lipoids were found in the urine only after the kidney had been previously damaged with uranium nitrate. These facts completely differentiating the finding of lipoids in the urine from the normal cholesterol metabolism of the body as evidenced in the blood stream would seem to link them closely with the etiologic complex of the diseases in which they may be found.

Miloslavich has classified these diseases under the following heads:

"1. Lipoid nephrosis of unknown origin (genuine lipoid nephrosis)

"2. Lipoid nephrosis of syphilitic etiology (secondary stage of syphilis)

"3. Subacute and chronic glomerulonephritis (combination forms of nephritis and nephrosis)

"4. Amyloid nephrosis with edema

"5. Grawitz tumor (so-called hypernephroma) of the kidney

"6. Lipoid carcinoma of the prostate"

He advises us that the micropolarscopic examination of a urinary sediment from these diseases may reveal the following morphologic elements:

"a. A minute, double refracting granule, either isolated or found in groups. (Spoken of in this paper as 'granule.')

"b. A cast, consisting mainly of anisotropic material, which is termed lipoid cast.

"c. Small epithelial cells, apparently desquamated cellular elements of the tubular apparatus of the kidney, which contain double refracting substances in their protoplasm. (Spoken of in this paper as lipoid epithelium.)

"d. Larger, foamy cellular elements, including anisotropic fat substances, derived from blastomatous growths in the genitourinary tract." (Spoken of in this paper as lipoid crystals.)

In a private communication[2] Professor Miloslavich has further advised us that the exact chemical constitution of these elements is not well understood, and that it is known to vary, though the constant constituent is cholesterol.

Our investigations conducted over a period of about two years have placed us in a position to add but little to this classification. The typical

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large lipoid granule under the low (10 × 10) power of the microscope with crossed Nicols reveals a characteristic polarizing cross-figure, somewhat resembling a black Maltese cross. When small this cross-figure may be barely discernible under the high (10 × 40) power. We also believe that there occurs a tiny granule discernible under the high power, the morphology of which, described in two planes only, resembles four diamonds with acute angled points placed in close juxtaposition. We have definitely identified this morphology in two specimens. The lipoid granules are not soluble by boiling the specimen for several minutes, though this procedure seems to reduce their anisotropic property. They are not immediately soluble in equal parts of urine and any of the following reagents: (a) 3.5 per cent neoarsphenamine solution. (b) N/10 sodium hydroxide. (c) 5 per cent acetic acid. (d) 6 per cent sulphuric acid.

The lipoid epithelial cell is the most frequently found lipoidal element, having been observed in 41 per cent of our 152 positive specimens, or 5.5 per cent of our 1146 valid examinations. The anisotropic phenomenon exhibited in this element may at times be but very faintly pronounced, its degree depending undoubtedly upon the concentration of lipoid substances in the protoplasm.

At this point it might be well to state that for the purposes of our investigation one of the laboratory microscopes was equipped with Zeiss polarizing attachments, and the principles of procedure as laid down in Simon H. Gage's textbook were followed throughout. The selenite test plate was not used. Its use is to be recommended, however, in any further investigation or routine work, as it will render more positive the identification of but faintly anisotropic substances.

The lipoid cast can be differentiated from the common granular cast only on the basis of the anisotropic phenomenon by the use of polariscopic attachments. The double refracting substance in general seems to be evenly diffused through the body of the cast, as it is in the lipoid epithelial cell, though we have observed a specimen in which the coarsely granular casts seemed to consist largely of small lipoid granules, the characteristic Maltese cross figure being distinctly visible under crossed Nicols.

The foamy elements, which we have designated as lipoid crystals, are quite characteristic in appearance. Under crossed Nicols they seem to contain spots of a much more highly refractive nature than the body of the element, the whole being of a somewhat cellular type of structure considerably larger than an epithelial cell.

We have also identified in a very small number of specimens, 3 per cent of the positives, less than 1 per cent of the total, an anisotropic pus cell. The greatest numbers of these were observed in two different cases of diabetes complicated by pyelitis, which suggests the possibility of the existence of a complex of this type.

In general our findings, published in Tables I to IV, prove the truth of Miloslavich's assertions that doubly refracting lipoidal substances may be found in other conditions but are particularly characteristic of the degenerative diseases of the kidney. It has not been our opportunity to examine speci-

TABLE I

869 SURGICAL SPECIMENS

	NEGATIVE	PER CENT	INVALID	PER CENT	GRANULES	PER CENT	CRYSTALS	PER CENT	CASTS	PER CENT	EPITHELIUM	PER CENT	PUS	PER CENT	TOTAL POSITIVES	PER CENT
Appendectomy	262	85	67	18	12	4	9	3	2	—	22	7	—	—	45	15
Salpingectomy	38	76	7	12	2	4	—	—	1	2	8	16	1	2	12	24
Miscellaneous laparotomy	58	82	14	16	6	8	1	—	1	—	5	7	—	—	13	18
Traumatic cases	36	100	11	23	—	—	—	—	—	—	—	—	—	—	0	0
Infections	23	96	5	17	—	—	1	4	—	—	—	—	—	—	1	4
Kidney	12	100	—	—	—	—	—	—	—	—	—	—	—	—	0	0
Gall bladder	19	73	6	19	5	19	1	4	1	4	—	—	—	—	7	27
Thyroid	11	85	3	19	—	—	—	—	—	—	2	15	—	—	2	15
Tumors	23	82	3	10	1	4	—	—	—	—	4	14	—	—	5	18
Eye, ear nose, and throat	38	97	9	19	1	3	—	—	—	—	—	—	—	—	1	3
Gynecologic	70	96	6	8	—	—	—	—	—	—	3	4	—	—	3	4
Genitourinary	12	92	4	24	—	—	—	—	—	—	1	8	—	—	1	8
Plastic (herniotomy)	12	80	4	21	3	20	—	—	—	—	—	—	—	—	3	20
Minor	15	83	5	22	1	6	—	—	—	—	2	11	—	—	3	17
Totals	629	86.8	144	17	31	4	12	2	5	—	47	6	1	—	96	13.2

TABLE II

501 MEDICAL SPECIMENS

	NEGATIVE	PER CENT	INVALID	PER CENT	GRANULES	PER CENT	CRYSTALS	PER CENT	CASTS	PER CENT	EPITHELIUM	PER CENT	PUS	PER CENT	TOTAL POSITIVES	PER CENT
Acute infections	94	87	24	18	4	4	—	—	8	7	1	1	1	1	14	13
Degenerative																
Kidney	45	71	5	7	14	22	—	—	4	6	—	—	—	—	18	29
Heart	31	91	12	26	—	—	—	—	1	3	2	6	—	—	3	9
Liver	1	100	—	—	—	—	—	—	—	—	—	—	—	—	0	0
Spleen	1	100	—	—	—	—	—	—	—	—	—	—	—	—	0	0
Metabolic																
Pancreas	120	95	17	12	4	3	—	—	—	—	—	—	2	2	6	5
Thyroid	6	75	1	11	—	—	—	—	—	—	2	25	—	—	2	25
Blood	1	100	—	—	—	—	—	—	—	—	—	—	—	—	0	0
Chronic infections	20	83	4	14	2	8	1	4	1	4	—	—	—	—	4	17
Nervous and mental	42	93	5	10	—	—	—	—	—	—	3	7	—	—	3	7
Gastrointestinal	11	85	3	19	1	8	—	—	—	—	1	8	—	—	2	15
Metallic and food poisoning	3	100	3	50	—	—	—	—	—	—	—	—	—	—	0	0
Totals	375	87.8	74	15	25	6	1	—	14	3	9	2	3	—	52	12.2

mens from the disease types 1, 2, 5, and 6 of the Miloslavich classification, nor to attempt to differentiate between a cardiac and a renal dropsy by means of the micropolariscope.

As previously indicated our investigations have been conducted by means of Zeiss polariscopic attachments, and without the use of the selenite plate. The micropolariscopic examinations were carried out on the sediments remaining in the centrifuge tubes after the completion of the routine chemical and microscopic examinations. To assure ourselves of the validity of our technic, as well as to familiarize ourselves with the morphology of the elements to be sought, an intensive preliminary study of three months' duration was made during which period 225 specimens were examined. No record of coincidental findings nor diagnoses was kept. Thereafter a complete record of the chemical and microscopic findings was kept, and the diagnosis in each case obtained from the chart while the patient was still in the hospital. These latter were subsequently checked in doubtful instances against the patient's index file of the hospital giving the patient's final diagnosis on discharge.

Very early in our investigation there became apparent an inherent weakness of the micropolariscopic examination which cannot be readily avoided. This arises from the presence in a large percentage of specimens of numerous crystals of comparatively no significance which are all highly anisotropic. It has been found possible to identify lipoidal elements morphologically among such crystals, but where they are present in any number they flood the field of the microscope with crossed Nicols with light to such an extent that the identification of lipoids among them would be well-nigh impossible, and it became necessary to throw out such specimens and record the polariscopic examination as "invalid." Twenty-two per cent of our examinations were invalidated in this way, bringing our total number of valid examinations down to 1146.

Another weakness of the polariscopic procedure is suggested by our investigation rather than demonstrated. Approximately 95 per cent of the lipoidal positives observed have come from acid or neutral specimens. The question arises: Does the chemical complex resulting in the crystallization of a cholesterol ester in the human organism require an acid environment, and may not these substances be present in urine in solution forms not detectable by the micropolariscope? The evidence would seem to indicate that it may. Of 20 specimens examined coming from diseases of the Miloslavich classification but one was alkaline, and this one did not show presence of any lipoids though all other seven of the eight examined from the same case were positive micropolariscopically for lipoid granules or lipoid casts or both.

Our observations do not throw much light on the physiologic chemistry of the processes by which the lipoids are produced and eliminated in the urine. One case of acute terminal nephritis of mixed type in a child is worthy of mention. The urine was positive only for albumin and blood for several days. Later, casts began to appear in increasing quantities. The micropolariscopic examinations at this time were negative. The quantity and character of the casts changed very markedly over a period of several days, the number increasing and the casts becoming more and more coarsely

TABLE III

100 OBSTETRIC SPECIMENS

	NEGATIVE	PER CENT	INVALID	PER CENT	GRANULES	PER CENT	CRYSTALS	PER CENT	CASTS	PER CENT	EPITHELIUM	PER CENT	PUS	PER CENT	TOTAL POSITIVES	PER CENT
Uncomplicated	24	100	2	9	—	—	—	—	—	—	—	—	—	—	0	0
Toxic	40	81	4	8	3	6	1	2	1	2	3	6	1	2	9	19
Surgical	20	95	—	—	—	—	—	—	—	—	1	5	—	—	1	5
Totals	84	89.4	6	6	3	3	1	1	1	1	4	4	1	1	10	10.6

TABLE IV

COINCIDENCE OF 152 LIPOID POSITIVE SPECIMENS

	GRANULES	PER CENT	CRYSTALS	PER CENT	CASTS	PER CENT	EPITHELIUM	PER CENT	PUS	PER CENT	TOTAL POSITIVES	PER CENT	PER CENT POSITIVE OF TOTAL EXAMINED
Acid or neutral	49	32	12	8	18	12	61	40	5	3	145*	95	13
Alkaline	9	6	2	1	2	1	2	1	—	0	15	10	2
Chemical and microscopic negative	17	11	7	5	—	0	26	17	—	0	50	33	5
Albumin or casts	27	18	5	3	20	13	14	9	1	—	67	44	6
Albumin and pus	7	5	—	0	1	—	12	8	4	3	24	16	2
Pus	9	6	2	1	3	2	7	5	1	—	22	14	2
Sugar	3	2	2	1	1	—	5	3	1	—	12	8	1
Acetone-postanesthetic	14	9	2	1	1	—	17	11	—	0	34	22	3
Acetone-toxic	2	1	—	0	—	0	2	1	1	—	5	3	1
Red blood cells	14	9	1	—	—	0	3	2	—	0	18	12	2
Bile	—	0	1	—	2	1	2	1	—	0	5	3	1

*Includes 8 specimens containing more than one lipoidal element—5.3 per cent of the positives.

granular in character. Waxy casts then appeared and for several days their proportion increased, the proportion of granular casts subsiding. The lipoids did not appear until three days after the first appearance of the waxy casts, fourteen days previous to the death of the child. The urine thereafter remained positive for the typical lipoid granule for several days until no further specimens could be obtained, the patient developing a complete anuria.

It is our opinion that the lipoid granule is a finding of graver significance than either the lipoid epithelial cell or the lipoid cast. It is reasonable to suppose that a small excess of lipoidal substances in the kidney might be eliminated in the protoplasm of epithelium and casts, whereas the chemical complex resulting in the production and elimination of the formed granule would be of a more serious nature. This hypothesis would seem to be borne out by the findings in the surgical group of specimens, under which the tumors, thyroid surgery, gynecology, and salpingectomy subgroups all show the highest percentage of the lipoidal positives in the form of lipoid epithelium. The appendectomy, miscellaneous laparotomy, genitourinary, and minor surgery subgroups also show a high percentage of lipoid epithelium compared to other lipoidal forms.

The most interesting finding in the surgical group is the fact that the small group of kidney surgery specimens was entirely negative for lipoids of any form, while the gall bladder surgery group showed a very high percentage of specimens positive for the granule. The total positive finding of 27 per cent for this group represents in fact the closest approach of any group to the maximum finding of 29 per cent in the degenerative diseases of the kidney, a matter which we consider of some significance. Coupled with the experimental fact quoted by Miloslavich to the effect that hypercholesterolemia of itself does not result in the elimination of lipoids in the urine until after the kidney has been artificially damaged by the injection of a poison, it would seem to postulate the presence in the kidney in these degenerative diseases of some substance derived from source or sources exterior to the kidney itself, which substance can be caused to appear in the kidney by surgical trauma of the gall bladder.

The positive findings in plastic surgery (herniotomys) also were high. It is to be regretted that the number of specimens available from these two groups was not sufficient to carry greater weight.

The findings in the group of specimens from medical cases serve only to confirm Miloslavich's assertions with regard to the importance of urinary lipoids in degenerative diseases of the kidneys, the incidence in this subgroup being 29 per cent. A high incidence of 25 per cent was also found in diseases of the thyroid, the finding consisting, however, of but two specimens containing lipoid epithelium out of a total of eight examined.

The obstetric group findings serve only to substantiate the fact that in normal pregnancy no injury to the kidney is to be expected.

We have already emphasized the fact brought out by Table IV of coincidence of 152 positive specimens that 95 per cent of these have been found in acid or neutral urines. This table shows that the micropolariscopic examination may be expected to disclose positive findings in 33 per cent of

total lipoid positives or 5 per cent of all pathologic specimens where all other findings are negative. This percentage of total specimens is comparatively small, and in view of the high probability of the examination being invalidated we cannot agree with Miloslavich that the micropolariscope should be employed as a routine procedure except in certain selected groups of cases from which further information is to be desired. The table also shows that lipoids detectable by micropolariscope may be found in conjunction with all of the other substances of a positive nature commonly looked for in urine, being associated in greatest frequency with albumin and casts.

SUMMARY

One thousand, one hundred and forty-six valid routine micropolariscopic examinations of pathologic urine have been completed and the results have been tabulated. A general discussion of the incidence of lipoids in urine and their diagnostic significance has been given. A tool has been used which has disclosed its own weakness, but has brought forth information which demands to be checked by other means.

CONCLUSIONS

1. The incidence of lipoidal substances detectable by the micropolariscope in pathologic urine is approximately 13.3 per cent, the frequency of their occurrence being comparable to that of the finding of casts, and the weight of their diagnostic significance being comparable if not greater.

2. It seems probable that the micropolariscopic examination is invalid in a large proportion of specimens and that the true incidence of the lipoids may be much higher than indicated.

3. Evidence is offered suggesting the possibility that conditions of the gall bladder of a pathologic nature, not at all clearly understood, may bear an etiologic relationship to the degenerative diseases of the kidney, and further investigations of the occurrence of lipoids in urine from cases of gall bladder as well as hernia surgery are to be desired.

4. The physicians should make an urgent demand of the biologic chemists for a simple chemical test for the detection of lipoids in urine. All that we in the routine clinical laboratory can do is to record and tabulate and report what we have observed.

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MERCURY POISONING; ITS CLINICAL DIFFICULTIES AND ITS PATHOGENESIS

Report of Two Cases of Brown Sublimate Kidney*

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ACUTE mercurial poisoning is a familiar and well-defined syndrome, with characteristic history, symptomatology, laboratory findings and pathologic picture. But not always is a history available. The poisoning may be accidental or homicidal, and neither the victim nor attendants ever suspect the correct cause. It may be suicidal, and the patient carefully withhold the information which would institute life-saving remedies. As a frequent penalty of ill-advised therapeusis, or of attempts at contraception or abortion, mercury poisoning is admitted often only with faint-hearted reluctance, or not at all.

Lacking a history, one may be led to the correct diagnosis by the typical clinical picture of the acute cases. But the direct and differential diagnosis is not always as simple as textbooks outline. The cases are rare. Unless one is unusually alert for it, without a history, mercury may never even be considered. When it is, its consideration may be submerged by more common syndromes which closely simulate it.

Laboratory methods fail us, usually because we do not even think to use them. Cases are often unwittingly stumbled upon during routine chemical examinations. Nor does every finding of a trace of mercury mean mercury poisoning. Industrial or accidental exposure to minute quantities, even amalgam fillings may cause the appearance of small amounts of mercury in the excreta, without any serious symptoms being attributable to it.[1] An adequate amount of mercury must be found to incriminate it as the etiologic factor.

Even the pathologic picture is subject to puzzling variation. Mercury works its fatal way in devious manners. It may rapidly cause death by direct cardiomuscular damage with circulatory failure and high anhydremic leucocytosis, before there has been time for appreciable kidney damage.[2] Death may result from slower but direct mercurial action on all the organs, from the incidental enteritis or other infections, or starvation or dehydration acidosis, or from an accidental infection without sufficient renal damage ever appearing. Massive renal damage with true uremia is, however, the lethal factor in typical cases.

The histologic picture depends on the dose, the interval after which death occurs, the associated pathologic changes and other less well under-

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stood factors. But the time sequence of changes has been very definitely worked out.[3] With a given fatal dose, and a known time after ingestion, and the associated changes determined, the microscopic picture may be more or less accurately anticipated. Thus mercury offers several varieties of renal picture, yet each is definitely established. These changes are not absolutely specific, for close resemblances occur with other poisons, such as bismuth, uranium, chromium, B-naphthol, sulphuric acid, oxalic acid, and hydrochloric acid. But they are sufficiently characteristic so that, with the slightest circumstantial support, they deservedly claim the name of "mercury kidney."

As a diagnostic aid, the microscope has therefore proved of great value, particularly when the diagnosis has slipped through the preceding elements in diagnosis. In two cases at the Cook County Hospital, in which a history of mercury was never obtained, and the clinical picture evaded diagnosis, microscopic examination led to confirmatory chemical tests which established it at least postmortem. Both recalled attention to several interesting details in renal pathology. Neither presented the usual picture associated with mercurialism. In each, the obscure clinical picture, reanalyzed, was secondarily clarified.

CASE 1—

Clinical History.—A white girl, seventeen years old, entered the hospital with an Admitting Room diagnosis of pelvic peritonitis. She stated that she had felt quite well up to four days before, when at midnight she was suddenly seized, for no apparent reason, with a severe, sharp pain. This pain began in the left pelvic region and radiated to the right side of the abdomen. She vomited several times, and once on the first day vomited blood. There was no desire for food, swallowing gave her pain, and there was pain in the epigastrium. She stopped urinating about sixteen hours after the onset of this attack. The anuria, the abdominal pain, and the vomiting persisted. She could take nothing at all by mouth. The pains grew worse.

The past history only added confusion. The patient had had "sleeping sickness" four years previously, and since then many fainting and dizzy spells. The mother volunteered that she had really been ill on and off for three years with vague abdominal pains for which an appendectomy had been fruitlessly done. She stated also that the patient had often had such urinary disturbances, and on a few occasions could not urinate for three or four days. She insisted that the present condition had all come on after drinking some ginger ale, of which she herself had partaken without harm.

The patient's menstrual history was normal. There had been a slight leucorrhea for a year, but no pregnancies or abortions. She firmly denied any medication or poisoning.

Physical Examination.—She was fairly well nourished. Her blood pressure was normal, 120/75, her pulse rate 90. Her breath was foul. Lips, teeth, tongue, and pharynx were dry and coated.

Heart and lung findings were altogether negative, and there was no edema.

The abdomen and genitalia were very tender, wherever touched. The hymen was deflorated. Vaginal examination was negative.

Catheterization of the bladder yielded only a few cubic centimeters of dark, bloody urine with a few epithelial cells. Cystoscopy was negative.

She suffered a rather severe diarrhea with loose, black stools which gave a four-plus reaction for blood.

Laboratory Examination.—The leucocyte count was 11,500 with 85 per cent polymorphonuclears. Temperature and respiration were normal. The blood Wassermann was negative. On the day of entrance, the urea nitrogen was 120 mg. per 100 c.c., creatinin 12.4, cholesterol 200 and carbon dioxide combining power 46 volume per cent.

Clinical Diagnosis.—Urinary suppression, the etiology of which could not even be ventured.

Further Course.—Treatment of the anuria was pushed desperately. Hot packs were applied to the lumbar region. She was given daily hypodermoclysis of 3000 c.c. of normal saline solution, and 1000 to 1500 c.c. of 10 per cent glucose intravenously with insulin. Sweating was tried, gastric lavage was done. Morphine was given to quiet her delirium and restlessness.

The second day after entrance, a decapsulation of the left kidney was performed. The stupor, the vomiting, the diarrhea, the anuria persisted. Her pulse became weak and thready. Digitose and pituitrin were accordingly given. Continuous proctoclysis was instituted. Edema never appeared though she received a tremendous quantity of fluids. The blood pressure rose to 160/80.

The blood chemistry remained about the same despite all treatment. On the tenth day of her illness, she passed 160 c.c. of turbid urine containing many red blood cells. On the eleventh day she passed 210 c.c. of similar urine, but on this day lapsed into deeper coma, and died.

Postmortem Examination.—The lips were crusted, the teeth were in fair condition. In the left lumbar region was a recent surgical wound, 15 cm. long. Though subcutaneous edema was absent, there was a bilateral hydrothorax of 1000 c.c. of clear fluid in each plural cavity, hydropericardium, an ascites of 500 c.c., and marked edema of the lungs, liver, and brain. There were striae albicantes in the lower abdomen, and an ancient appendectomy scar.

The heart was of normal weight, 300 gm., but above the valves was the longitudinal wrinkling of a syphilitic aortitis. In the urinary bladder was 300 c.c. of yellow cloudy urine which gave a positive albumin reaction. There were punctiform hemorrhages in the mucosa. The uterus was hypoplastic, only 4 cm. long. Its mucosa was covered by soft blood clots. The left ovary contained a corpus hemorrhagicum 10 mm. in diameter.

The stomach and small intestine were but little changed. The gastric mucosa was but slightly congested, the ileal mucosa pale. The cecal mucosa however was deeply injected, dark red grey with several transverse superficial ulcerations. These ulcers had indented edges, a light yellow grey base, and measured from 1 to 3 cm. in diameter.

The right kidney alone weighed 300 gm., was soft and wet. The capsule stripped readily leaving a smooth, pale, grey-red surface studded by a few minute deep red areas. Surfaces made by section showed the cortex 10 to 15 mm. thick, pale grey-red with obscure markings. The medulla was darker red. The left kidney weighed 320 gm. The capsule had been previously removed and on the convex margin were two longitudinal incisions extending into the kidney substance for a distance of 1 cm. On the anterior aspect, near the hilus, there were two irregular deep red areas with pale grey centers, 25 and 15 mm. in diameter, extending 10 mm. into the parenchyma.

Kidney.—There was very marked dilatation of the convoluted tubules. The epithelium was very low. In some of the tubules it was flat to moderately high cuboidal, with oxyphilic granular cytoplasm. Some of these cells were vacuolated. The nuclei were normal in number, and varied from large vesicular to small pyknotic ones. Most of the tubules however, were completely relined by flat to low cuboidal cells with basophilic, homogeneous cytoplasm, indistinct cell membranes, and very numerous, large nuclei, rich in chromatin, which bulged into the lumen. An occasional mitotic figure was seen among these nuclei. The lumen of the tubules contained fine branched threads of a pale stained material, and single desquamated vacuolated cells. Some of the tubules were filled by fibrinoid material, others contained homogeneous hyalin material, and a few showed erythrocytes. No calcium concretions were to be seen.

The Bowman's spaces of the glomeruli were wide and empty. The parietal epithelium of some was swollen, with numerous proliferated nuclei. Many of the capillary tufts were narrow, with the intraglomerular portion of the afferent vessels distended but empty. The interstitial tissue was diffusely increased, loose, strikingly edematous. There were occasional, single lymphocytes and plasma cells, but no accumulations thereof. The medulla was markedly hyperemic.

The whitish and dark red areas away from the surgical incision in the left kidney showed a diffuse necrosis of the renal parenchyma, with extensive recent extravasations of blood. Here and there, the necrotic tissue was infiltrated with polymorphonuclear leucocytes. The arteries in the necrotic area were partly filled by fibrin. Specimens were then submitted to the coroner's chemist, Dr. R. W. Webster.

Chemical Examination—

0.00541 gm. of mercury (calculated as bichloride of mercury) per 140 gm. of kidney tissue.
0.00157 gm. of mercury per 290 gm. of liver tissue.
0.0005 gm. of mercury per 219 gm. of heart tissue.
0.00015 gm. of mercury per 213 gm. of uterus tissue.

Anatomical Diagnosis was essentially:

Mercury-bichloride nephrosis (atypical form), with ulceropseudomembranous typhlitis.

CASE 2—

Clinical History.—A white woman, twenty-eight years old, was admitted to the gynecologic service with a diagnosis of bilateral salpingitis. Nine years previously she had given birth to twins, and one year later to a third child. For eight years her menstrual periods were uninterrupted, and symptomless except for a thick white discharge and slight premenstrual cramps. The menses were always regular until July 21, 1929, when they stopped abruptly.

She missed the August period. Shortly after this, about September first, for no reason at all she insisted, she began to have pains in both lower quadrants, especially the right, as well as pain in other parts of the abdomen and over the sternum. After a few days, there began spells of vomiting. She would vomit seven or eight times a day. This persisted, so that for four or five weeks she was unable to hold any food down at all. In the last week before entrance she was unable to retain even water.

During these five weeks she salivated excessively so that she was always expectorating or wiping her mouth. She suffered from terrific thirst, and wanted to drink all the time. She was constipated, but noticed black, tarry stools at times. Since the beginning of her illness there had been a marked oliguria. She wondered that she had to urinate only once during the day, and only once at night. A few times blood was seen in the urine.

Physical Examination.—She appeared to be acutely ill. She was quite obese, had a blood pressure of 118/70, a very rapid pulse of 160, an occasional chill with a temperature which never exceeded 100° F. and was usually normal. The face was flushed. A distinct jaundice was present. Heart and lung findings were negative. The area of liver dullness appeared to be decreased.

On vaginal examination, both adnexa were tender but contained no masses. The vaginal mucosa showed the deep purple of Chadwick's sign, and Hegar's sign was also positive. The uterus was soft, and enlarged to the size of a two and one-half months' pregnancy. The cervix was eroded, with many nabothian cysts.

A catheterized urine specimen was bloody and opaque. The white blood cell count was 12,350/mm.,^{3} the erythrocyte count was 4,900,000, the hemoglobin 90 per cent. The Wassermann reaction was negative.

Clinical Diagnosis was tentatively:

1. Pregnancy with pernicious vomiting.
2. Pyelocystitis, and
3. Endocervicitis.

In attempted treatment of the pernicious vomiting, all oral intake was stopped, hypodermoclysis and proctoclysis were instituted. But the vomiting continued, and the patient was transferred to the obstetric service in contemplation of more radical therapy.

Further Studies.—A blood chemistry report was returned with a urea nitrogen of 75.5 mg./100 c.c. of blood, a creatinin of 2.2, a cholesterol of 178, and an icterus index of 30. A phenolsulphonephthalein test returned less than 15 per cent of the dye in three hours. In

the Fishberg test, only 200 of the 1000 c.c. of water given were excreted within four and one-half hours. The highest specific gravity of the urine was 1.016. It still contained albumin and casts, bile, blood, and white blood cells.

Ophthalmoscopic examination showed numerous petechial hemorrhages in both fundi. She was evidently quite toxic. Her lips were dry and parched, with beginning sordes. Her gums were injected and there was a foul odor to her breath. Yellowish-white membranes were seen over her injected pharynx. Both lumbar regions were tender.

Clinical Diagnosis was then:

1. Pregnancy with toxemia.
2. Acute nephritis or acute yellow atrophy of the liver to be considered.

The patient improved somewhat for a time, and a cystoscopy was done. Its findings were negative. Even the phenolsulphonephthalein appeared from each ureter in normal time, but the percentage returned was very low. Then she began to complain of epigastric distress. Her vomiting persisted despite all treatment. Under spinal anesthesia, a therapeutic abortion was done.

There was still not the slightest suspicion of poisoning. The husband then admitted that just before the onset of her illness, the patient had taken quinine and ergot pills "to help the lower abdominal pains restore her menses." The mother added that she had also taken some "Gold Medal pills,"* and that these had precipitated the vomiting.

There was no improvement in her general condition. Her azotemia rose to a urea nitrogen of 175, a creatinin of 16. A serosanguinous discharge issued from the vagina. All supportive measures failed, and she died about two months after the onset of her illness, five days after the therapeutic abortion. After three weeks of hospital observation, the diagnosis was still undetermined.

Postmortem Examination.—There was a slight, diffuse icterus. The teeth were in good condition. Colostrum was expressible from both breasts. The ankles were slightly edematous. The heart was of normal weight, 255 gm., but the myocardium was softened by a severe parenchymatous degeneration. The spleen and liver were similarly softened by cloudy swelling. The liver was 1920 gm. in weight. A thick, tarry, black bile filled the gall bladder. The intestines were grossly normal. The brain substance was edematous, the leptomeningi pale.

The uterus was enlarged to four fingers above the symphysis. It was 12 cm. long, 9 cm. wide and 5½ cm. anteroposteriorly. Its wall was 18 mm. thick, pale greyish-brown and very soft. The cavum uteri was lined by soft, adherent, light pink-grey to yellowish-grey material. In the lower uterine segment and cervix there was a light yellow, soft membrane firmly adherent to the wall. The right ovary contained a 10 mm. corpus luteum.

The kidneys together weighed 580 gm. Their consistency was diminished. The capsule stripped readily, leaving a light brown, smooth surface. The cortex was 9 mm. thick, its markings obscure. The medulla was purplish grey.

Microscopic Examination.—Kidneys: This furnished the first clue to the correct diagnosis. The convoluted tubules were seen to be markedly dilated. Many of them were lined by low-epithelium which showed no intercellular membranes and was markedly basophilic. Their nuclei were very numerous, lay close together and often bulged into the lumen. In places, small fat droplets could be seen. These basophilic, homogeneous multinucleated tubules stood out sharply among the other tubuli contorti with their higher, finely granular, more oxyphilic cytoplasm and fewer and paler nuclei. The lumen of the latter tubules was filled by granular vacuolar débris.

In the glomeruli distinct swelling and nuclear proliferation of the parietal epithelium was readily observed. The Bowman's space often contained a vacuolar material similar to that seen in the convoluted tubules. Many of the connecting tubules contained clumps of pale erythrocytes or of erythrocytic débris. There was a very occasional connecting tubule which contained a small, spherical calcium concretion. The interstitial tissue was somewhat increased, loose, edematous. It had a few histocytes containing fine lipoid granules, but no cellular infiltrations.

*These contain ferrous sulphate, aloes and oil pennyroyal with no trace of mercury.

Liver: The centro-acinar liver cells and Kupffer cells contained yellowish-brown pigment granules.

Uterus: There was diffuse necrosis of the internal third of the wall, separated from the rest by a distinct zone of demarcation with numerous degenerated leucocytes. There were clumps of cocci in the necrotic area. Beyond the pyogenic wall, some of the lymph vessels were transformed into intramural abscesses.

Specimens were then submitted to the coroner's chemist.

Chemical Examination.—0.0044 gm. of mercury in 2.51 gm. of kidney tissue. No mercury was found in the liver.

Anatomical Diagnosis was essentially:

1. Subacute mercury bichloride poisoning.
2. Diphtheritic suppurative endocervicitis.
3. Postpuerperal suppurative endometritis.

Clinical Discussion.—The first case, an example of acute mercury poisoning, the second an example of subacute mercury poisoning escaped clinical diagnosis primarily for lack of history. The careful silence maintained by both patients with respect to poisoning proved an effective shield. Mercury was not suspected even enough to call for chemical tests antemortem.

That mercury was absorbed is certain, but how it was introduced is altogether obscure. Poisonings have been described by every possible route and orifice. Oral ingestion is the most common, but in neither of these cases was there sufficient local corrosion to establish or to even call attention to an oral route.

A particularly potent source of fatal and readily disguised mercury poisoning is the vaginal route. Numerous cases have been described. The mercury is applied usually as a contraceptive, or as an abortifacient or as more discreetly called an "emmenagogue." Even as a disinfectant douche, it may be rapidly absorbed with serious result. Le Doux,[5] Rynd[6] and others have therefore decried its free, even therapeutic prescription. Absorption from the vagina is rapid because of its rich lymphatic supply and acid reaction.[7] It is dangerously increased by the local hyperemia of hot douches in which it is used, by any local inflammation, by the irritation attending attempts at abortion, even by the hyperemia of a normal pregnancy.[8] For example, as a douche in chronic endocervicitis mercury may long be harmlessly used until the vaginal hyperemia of a supervening pregnancy causes toxic absorption. The vaginal introduction of mercury under these conditions bears an especially grave prognosis. It should be guarded against and watched for.

The average fatal dose, as given by Goldblatt,[9] is almost 2 gm. (28 grains), but less will suffice. The severity of symptoms is not proportional to the dose, because absorption and individual susceptibility are important variables.[1]

In retrospect, the symptomatology of both cases seems typical of an acute and of a subacute mercury poisoning. But no such syndrome was suspected before autopsy revealed the diagnosis.

FOUR STAGES

Landau and Fejgin[10] have outlined the clinical history into four typical stages with definite time sequence.

The first stage of local corrosion lasts from intake of the poison to the beginning of anuria, usually within twenty-four hours. There is severe local pain and burning, and other symptoms which vary with the route of introduction. In oral cases an early preliminary period of vomiting soon appears (within fifteen minutes to several hours). The sooner it begins after ingestion, the better is the prognosis.[9] In extraoral cases it is absent.

The corrosion symptoms subside in the second stage of anuria which lasts from the second to the seventh day or longer. Anuria lasting more than seven days, as it did in the first case, is uniformly fatal. The anuria is attributed to the loss of water by the vomiting and diarrhea which supervene, to the low blood pressure, and to the stuffing of renal tubules by desquamated tubular epithelium. Elwyn[11] ascribes it to constriction of the renal vessels by the tensed capsule of the swollen kidneys. This would support the rationale of therapeutic decapsulation. But Held[12] has indicated that those cases which appear brilliantly benefited by decapsulation are operated upon about the time when spontaneous recovery usually appears anyway.

Edema is absent, probably largely from lack of water. When it is supplied, effusions may appear. A marked azotemia rapidly develops. This is the result of two factors, the paralysis of kidney function which stops excretion, and the severe, toxic increased destruction of proteins by mercury all over the body.[13] In exceptional cases, the blood pressure is raised; usually it remains low. The retained products of metabolism themselves aggravate the renal and general damage.

If the patient lives to urinate again, the danger is not yet over. The stage of oliguria may go on even to a polyuria. With polyuria there may a steady decrease of the blood nitrogen retention as the patient passes through the fourth stage of recovery. But the oliguria may proceed to a fourth stage of increasing intoxication and death, with or without polyuria. For the concentrating ability of the kidneys may be so badly damaged that the hyposthenuric urine excreted is almost plain water. The tissue protein destruction continues in the third stage beyond the power of the crippled kidneys to cope with.

Corrosion gives the first symptoms, toxemia the second, and uremia closes the picture.

What factors will determine the return of kidney function and the cessation of tissue destruction, or otherwise, after a period of anuria is the most important problem in therapy. Very often the return of function is prevented and "eiweisszerfall" continues, not by reason of the original mercurial onslaught, but by previous renal damage, circulatory weakness, starvation and dehydration, exhaustion, by operative or medical insults, or by supervening infections to which these patients are especially susceptible. Thus the histologic picture of the cases described, particularly the second, indicated that recovery might have taken place if extrarenal factors including the operative procedures and the endometritis had not turned the scales. Supportive measures should therefore be the keynote of therapy.

Chemical examination offers the single most reliable antemortem diagnosis. Mercury can be detected in the urine, feces, gastric content or blood

with great facility.[14, 15] If only mercury is suspected, it is a simple matter to confirm or disprove it by a Reinsch test.

PATHOLOGIC DISCUSSION

Pathologic examination has often retrieved the diagnosis. Askanazy[16a] defined the kidney changes by their gross appearance into three stages:

1. Red primary stage.
2. Grey-white sublimate kidney.
3. Red sublimate kidney.

First Stage.—The red primary stage stretches through the first twenty-four hours after absorption of the sublimate, the period of local corrosion. It is only exceptionally seen at the autopsy table. The kidneys are red because of a toxic hyperemia which dilates all the capillaries of cortex and medulla. It begins with extreme rapidity, within five minutes after absorption.[4] The first parenchymal change is an edema and hyalinization of the glomerular tufts, and a thickening of the reticular substance which may be followed shortly by necrosis of the endothelial cells.[16b] The glomerular damage reaches its height within six hours but then quickly subsides.[17] Only traces of this glomerulo-nephrosis could be seen in the sections first described, in the swelling and proliferation of the Bowman's capsular epithelium.

Tubular injury begins almost as quickly, but continues to progress in severity. The site of election for the worst changes is the convoluted tubules of the third order, and its transitional part, with the descending limb of Henle's loops. The damage starts here and spreads in both directions.[18] Necrosis is soon seen here, but in the rest of the tubule only granular swelling and fatty or hyalin changes are all that is usually found.

It is only because of their precarious rôle as excretory organs that the kidneys are so severely implicated. If one ureter is previously ligated, the corresponding, nonfunctioning kidney suffers not the slightest damage from mercury absorption. Whether the mercury acts directly on the epithelium[19] or destroys it only secondarily by the anemia following a direct vascular spasm (Kaufmann) or a neurovascular irritation (Ricker), is in question. Stracke[20] experimentally made direct, binocular observations of such vascular disturbances. The marked idiosyncrasies which exist toward mercury speak more for its action on a sensitive neurovascular apparatus than on stable parenchymal cells.[21] On the other hand, Kosugi denies the appearance of such vascular changes. The extreme rapidity with which necrosis appears, the diffuse involvement of all tubules, the selection of a specific part of each tubule, argue more for a direct cellular damage by mercury than for a vascular mechanism. Be its mechanism what it may, the mercury necrosis pushes the kidney rapidly on into the second stage.

Second Stage.—The second stage of the grey-white kidney is mercury necrosis in full bloom. It is reached within twenty-four hours and lasts for seven days, or slightly longer, through the clinical period of anuria. It gives the usual picture illustrated in textbooks. The kidneys are swollen, pale grey or whitish grey. The striking pallor is the result of a diffuse oligemia

caused not by contraction or occlusion of the vessels but by their compression by the swollen tubular epithelium. A coagulation necrosis strikes the transitional part of most of the proximal convoluted tubules. Only a few are spared. The cells become small, are sharply outlined, with a homogeneous cytoplasm and pyknotic nuclei. Later, hyalin necrosis also appears giving swollen, granular, acidophilic cells with large vesicular nuclei. This is ascribed to acidosis or other extramercurial toxins for it may appear alone, even after all trace of mercury is gone, and prolong the damage.[22]

The necrotic epithelium of the convoluted tubules then separates from the basement membrane, and stuffs the lumen. This desquamation depends largely, according to Heineke,[19] upon actively regenerating cells from the basal epithelium. The young cells grow under the dead ones and push them out. They follow them into the lumen, surround the sequestrated cells like an involucrum, and grow in between them. They themselves may be caught at times in the coagulation or hyalin necrosis (see Gorke[24] and MacNider[25]). Many agree with Heineke that the young cells take active part in the resorption of the necrotic ones. Fahr[23] contests this view, holding that the regenerating cells do not digest the old ones, but only passively grow in, wherever they find room.

Regeneration.—The extraordinary rapidity and activity of these regenerative processes is the most specific characteristic of the sublimate kidney. It is the single and most constant diagnostic feature in this and later stages. Regeneration begins very early from the undamaged and even from the slightly damaged cells. Within seven days from ingestion of the poison, the necrotic cells may be desquamated and the tubules completely relined by a new growth.

The new lining is readily distinguishable from the old. The cells at first are flat. They are poorly separated from one another so that they form almost a syncytial ring. They are crowded strikingly with numerous deeply chromatinized nuclei which bulge into the lumen from the flat cells. The nuclei are so numerous that they seem to lie one on top of the other around the lumen. The glomerular capsular epithelium is similarly affected. The cytoplasm of the young cells is basophilic and homogeneous, light blue in sharp contrast to the purplish red of the mature or the light red of the necrotic cells. As the lining matures it grows taller and more regular, cell membranes become distinct, cytoplasm turns granular and oxyphilic, nuclei become less numerous. The young cells function poorly. As they mature, function returns.

Casts of necrotic cells still lie within the lumen of the tubules. Clearing the lumen by resorbtion of these casts, and pushing the epithelial relining to completion is the feature of the third stage.

Third Stage.—The stage of the red sublimate kidney begins usually after seven days, after the period of anuria. The redness is a result of a new hyperemia which is imposed upon the kidneys by two factors. One is the hyperemia of active regeneration. This almost always makes its appearance, but is moderate and lends only a light brownish color to the kidneys. In both of the cases described in this paper, only this color was reached. The other

factor is more variable. It is a hyperemia which is intimately concerned with calcification. It may be absent, as it was in these two cases, but when present offers to the kidneys the typical red color of the third stage.

Calcification.—Calcification of the necrotized cells first appears in the third stage. It affects only the cells involved in coagulation necrosis, not those involved in the hyalin necrosis. It affects particularly the cells disintegrating slowly in coagulation necrosis. A certain "vita minima" is necessary to induce the settling out of calcium, which completely destroyed cells cannot muster (Gorke[24]). Calcification, however, is not specific to the mercury kidney. It occurs to some extent in copper, iodine and phosphorous poisoning, in simple anemic infarcts, in nephritis, in nephrosclerosis, even in extrarenal disturbances. It may be slight indeed or absent altogether in proved mercury poisoning. Thus it is always absent until the third stage (about one week), and disappears as soon as the coagulation necrosis débris is cleared out. It is unlikely to appear where there is rapid necrosis; in some cases it never does appear.

Schmidt[26] has suggested as a likely factor in determining the presence or absence of calcification, the severity of intestinal tract damage. Calcium is normally excreted by the intestinal tract. If its mucosa is destroyed by mercury, the calcium is unloaded through the kidney. He contends therefore that calcification is seen in the kidneys particularly in those cases of mercury poisoning where the intestines are extensively involved. This is generally agreed. In argument he reports calcium concretions in the kidney in ordinary cases of dysentery. Only Lemke[21] dissents in reporting a case of mercury poisoning without colitis, without calcemia but with marked calcium deposit in both kidneys. Colitis is probably only one of the several factors involved. The first case described in this paper showed a marked colitis but no calcification at all; the second showed no colitis at all but a slight calcification.

A marked hyperemia attends the calcification of the necrotic cells. It brings in calcium with sufficient speed to enable it to accumulate about the dead cells. In turn the calcification arouses an active inflammatory response in attempts to evacuate it. The hyperemia increases and gives to the kidney of the third stage its typical deep red color. Leucocytes infiltrate the edematous interstitial tissue and even wander through the walls of the cast-stuffed tubules. They penetrate the masses of calcified necrotic cells and effect their resorption. The casts become smaller, are resorbed entirely or swept away, and the lumen is cleared. Simultaneously, the new epithelial lining matures and function is restored.

Slight calcification may be cleared up without an inflammatory reaction, with no interstitial infiltration and with only the hyperemia of active regeneration to give these third stage kidneys a light brown color. When however because of enterocolitis, calcemia, or for other reasons mentioned, any appreciable calcification occurs, there is marked interstitial infiltration and a severe hyperemia to give the more typical red sublimate kidney of this stage. Observations by Takahashi,[27] Schieck,[28] Hunter[29] and many others attest this fact. Volhard[30] thus distinguishes two types of mercury nephrosis

in the third stage, one without inflammatory reaction, and the more usual one with inflammatory reaction. Both of our cases fell in the first and more infrequent category, with little or no calcification, no interstitial infiltration and only light brown sublimate kidneys, instead of the usual red ones.

CONCLUSIONS

Two cases of fatal mercury poisoning are described, which clinically were obscure. Lack of history was the chief diagnostic handicap. It led readily to incorrect interpretation of clinical symptomatology which in retrospect was typical for an acute and for a subacute poisoning. So remote was the suspicion of mercury that chemical examination was not even tried.

Histologic examination retrieved the diagnosis in both cases. It demonstrated the extreme activity and rapidity of regenerative changes in mercury nephrosis. This lively regeneration is more specific for mercurialism than is calcification which indeed was slight or absent in both cases. The pathogenesis of mercury nephrosis is reviewed. Attention is called to the fact that when calcification fails to appear, inflammatory reaction in the kidneys is likewise absent. There are then no interstitial infiltration and insufficient hyperemia to produce the typical red sublimate kidney of the third stage. Both cases described lacked this inflammatory reaction, and are presented as examples of the less frequent brown sublimate kidney.

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DETERMINATIVE STUDIES OF AN UNDESCRIBED SPECIES OF GRAM-NEGATIVE CORYNEBACTERIUM (C. QUASISTERILUM)*

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THE sanitary engineer or surgeon may at times be confronted by the appearance of irregular and bizarre species in his bacteriologic studies of the water he is investigating or using. An orange chromogenic gram-negative bacillus was isolated from the sterile tap water system of the Urologic Clinic on April 18, 1929. Five subsequent attempts at short intervals to isolate this or other organisms gave negative results upon plain agar and dextrose-peptone broth infusion. The organism was first isolated from the water of cystoscopic examining rooms four and five. On June 19, 1929, the cultures from the sterile water in rooms three, four, and five were again positive for a gram-negative chromogenic bacillus. It developed that the growth potential of the organism was cyclic, the negative phase varying from six to eight weeks following the flushing of the sterile water system with live steam. The activity of the organism is revealed in the following studies and does not confirm the assumption of propagation by spores.

The cystoscopist is likely to encounter such organisms in the use of certain supposedly sterile waters. This appears especially true when stock distilled waters are used and taken directly from large supply basins, even though the latter are seemingly faultless. The elaborate distilling and storage plants for sterile-water tap systems are not beyond some critical comment and sterilization of water immediately before being used for cystoscopic examination and surgical procedures would appear to be ideal.

The pathogenicity studies of the organism here reported were consistently negative. Mice, guinea pigs and rabbits were used. This report is recorded as a guide to surgeons who may find this organism in their distilled water. They may consider it a nonpathogen in the usual sense but the question arises as to the possibility of implantation and growth upon an already diseased tissue.

The organism is an orange, chromogenic, aerophilic, mesophilic, nonmotile, gram-negative bacillus. Its normal habitat is water. The bacillus is medium sized, as a rule 0.5 by 3.0 μ, but many individuals are quite small and "diplococcoid" in appearance. Diplo forms are very common, while chain formation is fairly frequent in certain cultures. Filamentous forms are found in old cultures which also show false branching. Wadsworth and the India ink methods show no capsules. Flagella stains are negative. Möller's and Ziehl-Neelsen stains show uniterminal granules, usually subterminal but oc-

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casionally terminal. Spores are not observed. There is apparent swelling of the cell bodies at the granules. Longer forms, 5 microns, occur and may show central or excentric granules. The granules are metachromatic by Neisser's staining. Rarely a chain of granules occurs throughout the length of the longer forms.

The moist-heat resistance test confirms the absence of spores by the various spore-staining methods.

Extensive pathogenicity studies on mice, rabbits and guinea pigs give no consistent or conclusive reaction.

Cultural reactions are quite distinctive. The colonies tend to remain discrete, even on ascitic agar, upon which they grow more profusely than on plain agar. On the latter the colonies are usually larger but much less numerous than those resulting from a similar technic of inoculation upon ascitic agar. The colonies on plain agar are round or slightly oval with smooth edges and with a distinctly raised, well demarcated and more deeply pigmented central zone which is brilliant orange in color at ninety-six hours. The lighter color of the outer zone of the colonies is seen to be a bright yellow. The pigment is also well developed, as a rule, at seventy-two hours. The raised central zone occupies just about half of the total surface area of each colony.

The optimum temperature on plain agar medium is 37° C. The organism is relatively a slow grower, even on ascitic and reinforced media. It is even more retarded on plain agar at 25° C.

Acid formation is slow and occurs only on maltose, saccharose, raffinose, arabinose, and the hexoses. On dextrose broth there was one-plus acid, three-plus pellicle, and four-plus sediment at seven days of growth. At ten days the acid and growth appearance was approximately as at seven days, augmentation of either being difficult to make out. Other dextrose, galactose, and sucrose cultures showed acid formation varying, of course, with inoculation dosage. Thus there was often a faint trace of acid present at seventy-two hours, a heavy trace at ninety-six hours, a 1-plus acidity at seven days, 2-plus at two weeks, and 4-plus at four weeks. Pellicle formation occurred on liquid sugars, alcohols, and glucosides but was uniformly retarded. No gas was found on any of the fluid or solid media. Litmus milk was unchanged at five days.

Growth is more rapid and profuse on whole blood agar and potato than on plain agar. The gentian-violet potato colonies at one hundred and forty-four hours are larger than those on plain potato but are always less numerous, the dye restricting the growth of some individuals; the colonies measuring 4.0 to 7.0 mm. at one hundred and forty-four hours on gentian-violet potato, high and bulging above the surface of the medium. They are round or oval, moist and dirty yellowish in color.

Gelatin is very slightly liquefied at twenty days.

Starch is not hydrolyzed at three or seven days' growth.

Loeffler's serum shows a trace of proteolysis at twenty-eight days.

The organism is indol-negative. The acetyl-methyl-carbinol reactions were uniformly negative. Methyl red reactions were negative. Voges-Pros-

kauer tests were negative. Two methods were used under parallel test conditions to check the reduction of nitrates: (1) the test as advised by S. A. B. in "Standard Methods" and (2) the nitrous-acid-starch-iodide test. These tests were uniformly negative and there was no apparent formation of ammonia. The organism does not reduce urea and does not utilize citrate agar in its metabolism.

The bacillus, classified after the "Key for the Identification of Organisms of the Class Schizomycetes" of the Society of American Bacteriologists (1923), is found to be of the order "Actinomycetales," family "Mycobacteriaceae," genus "Corynebacterium." No tribe category is specified by the Bergey *Determinative Manual* under the Mycobacteriaceae.

The character distribution of the organism roughly parallels that of certain of the Bacillaceae, in so far as the fragmentary known "characters" of some of the latter are recorded, but it does not form spores and in most respects resembles the Mycobacteriaceae. The organism likewise resembles a species of Flavobacterium in many respects (Flavobacterium ovalis—Wright).

The bacillus under consideration, then, cannot with finality be designated as any one of the known species. Since this is the situation, it appears better to create the distinctive specific name, "Corynebacterium quasisterilum (Cooper)."

SUMMARY

Morphologic, cultural, chemical and pathogenic studies were made of a gram-negative bacillus isolated from the sterile water system of cystoscopic and treatment rooms.

Results of the above studies are recorded and a name applied for the organism, the "characters" of which do not appear in conformity with any available recorded determinative studies of the Bergey *Manual* or in the literature.

Stock cultures of the organism are preserved on conservation medium and will be supplied to laboratories upon request.

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THE HISTOLOGIC CLASSIFICATION OF CARCINOMA OF THE CERVIX AS REGARDS ULTIMATE PROGNOSIS*

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FROM the time that Virchow gave his conception of a malignant tumor as a growth made up of cells that differ from the normal cells of the body in their rapidity and manner of growth, attempts have been made to classify them based on their histologic characteristics. This was found to be unsatisfactory, and no prognosis could be offered from the histologic structure of the tumor; for example, it was found that the prognosis of the breast tumor did not depend upon whether it was classified as an adenomatous, scirrhous or colloidal tumor.

In 1921 Broders and MacCarty offered a classification based upon cell differentiation and reproductive ability of tumor cells. These criteria proved to give a far more satisfactory classification and better possibility of prognosis. On these principles, cancers of the fundus of the uterus have been classified by Mahle; those of the tongue by Simmons; those of the cervix by Martzloff; and those of the lip, skin and genitourinary organs by Broders.

Broders based his classification of epitheliomas of the genitourinary organs on a study of 473 cases observed in the Mayo Clinic from Nov. 1, 1904, to July 22, 1915. In 269 of these cases the lesion was in the cervix. He divides these neoplasms into four grades as follows: If the tumor contains about three-fourths differentiated and one-fourth undifferentiated epithelium it is graded 1; if the differentiated and undifferentiated elements are about equal, it is graded 2; while if it shows one-fourth differentiated and three-fourths undifferentiated epithelium it is graded 3; and if there appears to be no attempt at differentiation it is graded 4. When correlated with the clinical history, this grouping proves to be significant, for he finds that the total good results for all organs were 83.33 per cent in Grade 1, 45.90 per cent in Grade 2; 25 per cent in Grade 3, and 12.19 per cent in Grade 4.

We have applied Martzloff's criteria in classification of cancer of the cervix in a number of cases, and found that his classification in too many instances did not harmonize with the end-results. Therefore, we made a study of our cases to determine, if possible, better microscopic criteria as the basis for classification. From this study we have been led to alter some of Martzloff's criteria, and have arrived at a method of classification which gives more satisfactory agreement with the clinical history in some three hundred cases.

Specimens from cases of over three years' duration only were used. If the patient was still alive, it was assumed that she was free from the disease.

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The method of classification follows:

Two classes were made, "high" and "low" malignancy. In the "low" group we classified all of the tumors that tended toward keratohyaline formation, in which the cells tended to form keratohyaline material and epithelial pearls. The cells in these cases more or less closely resemble in appearance and arrangement the natural stratified and squamous epithelium of the portiovaginalis of the cervix, and are thus well differentiated. The cells are large, very much of the same size and clear. In many cases the tumor cannot be distinguished from ordinary epidermoid carcinoma of any mucous membrane. In this class we see many "pearls." The more "pearls" the less the malignancy. If a sufficiently large piece of cervix is given the laboratory,

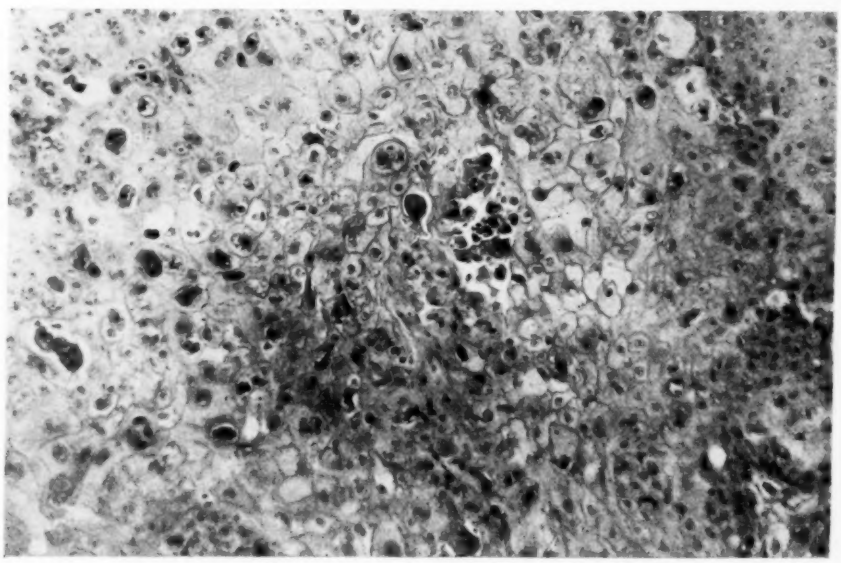

Fig. 1.—"Low type" cervical malignancy. There are attempts at keratohyalinization and "pearly body" formation. The individual cells are larger, clear and resemble the cells of the portiovaginalis.

the tumor sometimes assumes a warty appearance. There is also very little infiltration into cervical tissue.

The group that is classified as of "high" malignancy has the following microscopic appearance: The cells are of two types; the first type of cells are of uniform size, closely packed, with a clear cytoplasm and a small round nucleus containing very little chromatin; mitotic figures are few.

The second type of cell in this group resembles a spindle cell. They contain much chromatin, are closely packed, have many mitotic figures and there is never keratohyalinization or the formation of the so-called "pearly bodies." They have the appearance of cells found in the rodent ulcer, although when viewed under high power they are larger, show more mitotic figures, and contain much more nuclear chromatin. They invade profusely.

Fig. 2.—"High type" cervical malignancy. Spindle-shaped type of cell with dense chromatin, clear cytoplasm, relatively small nucleus and much mitosis.

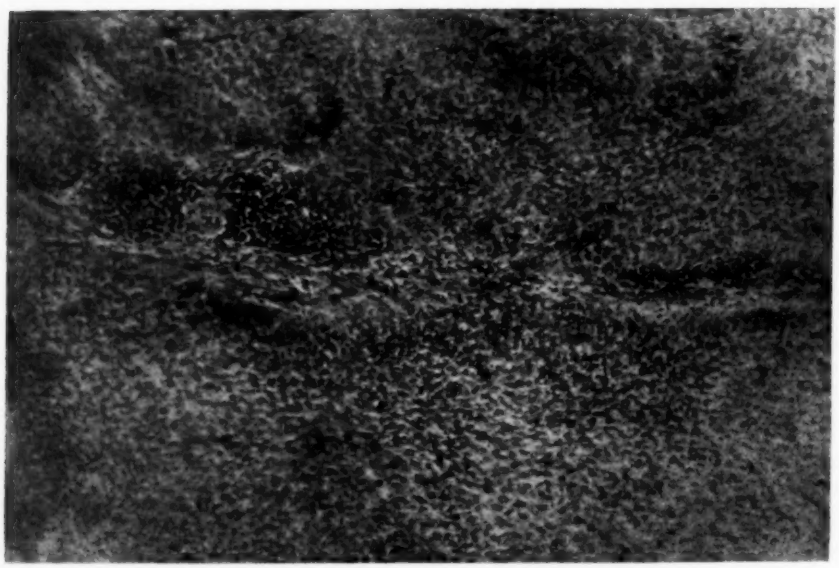

Fig. 3.—"High type" cervical malignancy. Round cells usually present with the spindle-shaped cells. These cells are quite large, show relatively little mitosis with little cytoplasm. A smaller amount of chromatin as compared to the spindle-shaped cells.

Generally both cell types are found in the same tumor, but many times only the latter type are seen. We have never found the first type of cell alone in the tumors studied.

We find another group comprising the adenocarcinoma, which formed a very small portion of our cases, and which were relatively nonmalignant in this series; we have accordingly confined our study principally to the squamous cell cancer.

The number of cases studied in each class are as follows: Out of 294 cases we found only 15 of "low" malignancy, 276 of "high" malignancy, and 3 of adenocarcinomas. Of the "low" malignancies all are living except 3 who died from other causes. Therefore, we conclude histologically that all cancers of the "low" malignancy group are of a relatively benign character clinically.

The "high" malignancy class gives an entirely different finding as to end-results. After the three-year period, all cases belonging to this class are dead from cancer. We therefore conclude that in this type of cancer a prognosis can very easily be made from the microscopic slide. It is evident from our findings that a section taken from whole tissue is much better than individual cell description. We have used Wright's new method of acetone fixation and find it wholly satisfactory for immediate diagnosis.

In the adenocarcinoma group we have only three cases and all are living after the three-year period. No attempt at classification is made because of the end-result obtained.

We have made no attempt to separate the prognosis or end-results in treated or untreated cases. The type of treatment, whether it was surgery, x-ray, or radium, gave little difference as regards end-results in our cases. We can see no relationship when the end-result is already known, although the type of treatment may have a great influence in early cases.

CONCLUSIONS

Two hundred ninety-four cases of cancer of the cervix were studied microscopically in an effort to determine the degree of malignancy and make some classification suitable to our needs, so that in the future some prognosis might be given. The microscope, according to our conclusions, is one of the most valuable assets in the determination of prognosis in malignancy. The squamous cell type of cancer was divided into two types of malignancy which we called "high" and "low."

The "low" group is made up entirely of large even cells, which have a tendency toward "pearl" formation. The cells as a rule are equal in size and do not infiltrate.

The "high" group is made up of spindle cells with much chromatin and a small nucleus. They are closely packed, giving the appearance of the ordinary basal cell carcinoma. This is the highest type or the most malignant type of cancer found. Along with this squamous cell we usually find a round cell with a clear cytoplasm and a very early chromatinized nucleus. This cell never invades tissue. The cell looks more or less normal. The round cell type of cancer is always mixed with the spindle cell type.

We have no accurate data upon adenocarcinomas. The three cases which we have had in our series of 294 are all living. Our experience with adenocarcinoma does not give any reliable data as to the degree of "high" or "low" malignancy of the disease process.

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THE INFLUENCE OF BLOOD GROUPS IN MALARIAL TRANSFUSIONS*

By E. M. Knights, Ph.D., Toledo, Ohio

DURING the year 1928 F. J. Farnell, psychiatrist for the Providence City Hospital, while treating cases of general paresis with injections of human blood containing malarial parasites, noticed that the period of incubation of malaria in the recipients varied and in some instances a single injection of from 2 to 5 c.c. of "malarial blood" failed to induce malarial symptoms in the recipient. Furthermore two pareties receiving blood from the same donor at the same time often differed widely in the incubation periods of their malarial symptoms.

Following a discussion of this situation the author asked for permission to type the donors of malarial blood and the recipient pareties, to prepare stained smears of the donors' blood and to prepare smears of the blood of the recipient pareties from time to time.

Early in our study it became evident that the question of blood groups was an important factor in determining the incubation period for malaria after the transfusion of small quantities of malarial blood.

Plasmodium vivax of benign tertian malaria was being used for this work and it is an accepted theory that in man the only time that the parasite is found free from the erythrocytes is during that stage of schizogony when the merocyte ruptures and the merozoites migrate to fresh erythrocytes.

If, due to an incompatibility of blood groups between donor and recipient, there is agglutination and lysis of the donor's red cells it is probable that only those malarial parasites which are in the merozoite stage and ready to infest new erythrocytes may survive. If, on the contrary, the donor and recipient are in the same blood group or in such a relationship that the erythrocytes of the donor are not agglutinated by the serum of the recipient we would expect a minimum incubation period.

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Another factor tending to influence the incubation period is undoubtedly the stage of development of the parasite in the blood of the donor at the time the transfusion is made.

There was, in nearly every case of successful malarial inoculation, a tendency for double tertian malaria to develop with chills occurring every twenty-four hours. In these double tertian cases two distinct stages in the development of the parasite were easily demonstrated in the majority of smears taken.

In cases where an incompatibility of blood groups existed and yet the period of incubation was shorter than might be expected it was often found that there was a predominance of full-grown merocytes in the blood of the donor and that the merozoites liberated by destruction of erythrocytes due to incompatibility were ready to attack fresh erythrocytes regardless of blood group.

The following twelve cases are illustrative of the findings in this study:

CASE NO.	RECIPIENT'S GROUP MOSS CLASSIFICATION	DONOR'S GROUP	INCUBATION PERIOD (ELAPSED TIME FROM INOCULATION TO FIRST RISE IN TEMPERATURE)
1	IV	IV	4 days
2	II	II	4 days
3	IV	IV	4 days
4	II	IV	10 days
5	IV	II	13 days
6	IV	II	9 days
7	II	III	11 days
8	IV	III	7 days
9	IV	II	7 days
10	III	IV	10 days
11	IV	III	12 days
12	III	II	No evidence after first injection. Reinoculated.
12	III	II	10 days

In February, 1929, there appeared in the *Archives of Pathology* an abstract of an article by G. Hopf on the "Significance of the Blood Group and of Plasmodium Sporulation on the Type and Incubation Period of Malaria." Hopf's* original article appeared in the October 12, 1928, issue of the *Münchener medizinische Wochenschrift*. In this article Hopf reported a study of 81 cases in which blood groupings were done before transfusions of malarial blood. His findings were the same as ours and certainly more conclusive. However, it is of interest to state that the case records at the Providence City Hospital will show that we started our work in May, 1928, without the knowledge that work was being done elsewhere along the same line.

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*Hopf, G.: München. med. Wchnschr. 75: 1755, 1756, 1928.

LABORATORY METHODS

A REDUCTION IN THE AMOUNT OF BLOOD REQUIRED FOR THE FOLIN MICRO BLOOD-SUGAR METHOD*

By Harold J. Jeghers, B.S.,† and Victor C. Myers, Ph.D., Cleveland, Ohio

AT THE time of the publication of Folin's micro blood-sugar method,[1] it occurred to one of us that the amount of blood employed could be reduced from 0.1 to 0.025 c.c. without materially changing the method. At first our hemoglobin pipettes were employed, but later we had pipettes constructed similar to the Folin pipette with the calibration mark at 0.025 c.c. We now use these smaller Folin pipettes for hemoglobin estimation. It is materially easier to obtain 0.025 c.c. of blood from a finger tip puncture, and we believe that our data show that the results are quite as satisfactory.

Observations on twenty-three miscellaneous hospital bloods are given in Table I. Determinations were carried out with the modified Folin micro

Table I

Comparison of Blood-Sugar Findings Obtained on Venous Blood with the Modified Folin Micro Method (Employing 0.1 and 0.025 c.c. of Blood) and the Folin-Wu and Benedict Methods

Specimen	Modified Folin micro method		Folin-Wu method	Benedict II method
	0.025 c.c. blood	0.1 c.c. blood		
	mg.	mg.	mg.	mg.
1	72	67	67	--
2	72	69	75	69
3	73	75	91	69
4	65	75	80	59
5	72	75	83	71
6	63	76	79	62
7	69	76	78	70
8	74	78	83	70
9	74	78	81	--
10	78	81	85	--
11	76	83	91	69
12	83	84	99	81
13	78	87	91	73
14	84	91	92	85
15	97	94	104	79
16	104	109	112	--
17	111	125	132	109
18	140	142	144	126
19	144	142	146	---
20	221	227	258	---
21	235	239	265	---
22	259	250	263	---
23	367	381	411	---

Last five specimens from diabetics. Analyses made July 2-25, 1929.

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†Crile scholar.

method[2] employing 0.025 c.c. and 0.1 c.c. of blood and by the Folin-Wu and Benedict II method.[3] With a few exceptions, the agreement between the two micro methods was fairly good, although for some reason the use of 0.025 c.c. of blood seemed to give slightly lower results, thus more nearly approaching the figures for the true blood sugar as indicated by the Benedict II method. A possible explanation for the slightly lower figures obtained when 0.025 c.c. of blood was employed is that the greater dilution of the blood with the tungstic acid solution may have led to a greater precipitation of the nonsugar reducing fraction. It is further possible that with substitution of a zinc precipitant for the tungstic acid the method might yield the true glucose content.

Table II gives a few comparative figures on finger blood. The agreement between the two methods is quite good, although with the smaller amount of blood the results are again slightly lower.

TABLE II

COMPARISON OF BLOOD SUGAR DETERMINATIONS ON FINGER BLOOD

BLOOD EMPLOYED	MG. SUGAR PER 100 C.C.						
	NO. 1	2	3	4	5	6	7
c.c.							
0.025	91	91	95	96	118	132	156
0.1	93	95	97	95	119	132	159

In the Folin micro method the proteins are precipitated with tungstic acid, the sugar in the supernatant solution after centrifuging oxidized with alkaline potassium ferricyanide, and the ferrocyanide produced measured colorimetrically as Prussian blue. This method is a very delicate one and furnishes an abundance of blue fluid for color comparison, even with 0.025 c.c. of blood. For this reason it is technically superior to any other micro method.

As originally described, the 0.1 c.c. of blood after dilution to 10 c.c. with the tungstic acid solution yields about 9 c.c. of extract, of which only 4 c.c. is used and ultimately diluted to 25 c.c. Our modification consists in doubling the preliminary dilution, i.e., 0.025 c.c. to 5 c.c. employing 4 c.c. of the extract and a final dilution of 12.5 c.c.

As pointed out by Folin,[1] it is essential that the micro pipettes should be perfectly clean to have them draw up the blood by capillary attraction. We always leave them in cleaning fluid overnight.

With the aid of a capillary pipette* calibrated to contain 0.025 c.c. of blood, allow blood after lancing finger tip to flow to mark or slightly above, adjust level of blood to mark, see that the outside of the pipette is free from blood, and immediately discharge contents into 5 c.c. of tungstic acid† in 15 c.c. centrifuge tube. Completely wash blood from pipette by sucking up blood several times, stir with the pipette, centrifuge, and pour the supernatant fluid into a clean dry test tube.

*Both the 0.025 and 0.1 c.c. pipettes have been made for us by Eimer and Amend, N. Y.

†Prepared by diluting 20 c.c. of 10 per cent sodium tungstate to about 800 c.c. with distilled water in a volumetric flask, adding 20 c.c. of 2/3 N sulphuric acid and diluting to volume.

Transfer 4 c.c. of the blood extract to a tube graduated at 12.5 c.c. To a similar tube add 2 c.c. of standard glucose solution* (0.01 mg. glucose per c.c.) and 2 c.c. of water (for diabetic bloods employ 4 c.c. of standard). To both tubes add 1 c.c. of the potassium ferricyanide solution† and 1 c.c. of the cyanide carbonate solution.‡ Heat both tubes in a beaker of boiling water for eight minutes. Cool in running water for one to two minutes. Now add 1.5 c.c. of the acid ferric iron§ to each tube, allowing it to run down the side of the tube to prevent foaming. Mix by gently rotating the tube, let stand for one to two minutes, add 5 c.c. of water, which should bring the volume to the 12.5 c.c. mark. Mix and make the color comparison.

If trouble is experienced in matching colors due to the yellow color of the ferricyanide (green), this can be compensated for by the use of a yellow light filter. In our work we have used the Klett biocolorimeter and have placed a strip of filter paper saturated with picric acid over the lamp window, following a suggestion made to us by Dr. Malmros before the publication of his paper with Dr. Folin. A suitable glass filter may also be used.

Calculation.—Since the standard contains 0.02 mg. glucose and the equivalent of 0.02 c.c. (⅘ of 0.025 c.c.) of blood is employed, the reading of the standard divided by the reading of the unknown times 100 gives the mg. of sugar per 100 c.c. of blood.

CONCLUSIONS

The Folin micro blood-sugar method is modified to employ only 0.025 c.c. of blood. It is much easier to secure 0.025 c.c. than 0.1 c.c. of blood from anemic and emaciated subjects, infants and children. Glucose tolerance tests may be run with less discomfort to the patient. It is a satisfactory routine method for diabetic patients.

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*This contains 0.01 mg. of glucose per c.c. in 0.025 per cent benzoic acid, and is prepared from a stronger solution containing 2 mg. per c.c. by diluting 200 times.

†Prepared by dissolving 1 gm. of c.p. potassium ferricyanide in distilled water and diluting to 500 c.c. (If chiefly diabetic bloods are to be examined, it probably is preferable to double the strength of the ferricyanide as Folin and Malmros now suggest.) The major part of this solution should be kept in a brown bottle in a dark closet, the portion in daily use also being kept in a brown bottle.

‡Prepared by dissolving 8 gm. of anhydrous sodium carbonate in 40 to 50 c.c. of water in a 500 c.c. volumetric flask with the aid of shaking, adding 150 c.c. of freshly prepared 1 per cent sodium cyanide, diluting to volume and mixing.

§Ferric iron solution prepared as follows: Suspend in a liter cylinder filled with water 20 gm. soluble gum ghatti on a copper wire screen just below the surface, and leave overnight (eighteen hours.) Remove the screen, and strain through the doubled layer of a clean towel. To this filtrate add a solution of 5 gm. of anhydrous ferric sulphate in 75 c.c. of 85 per cent phosphoric acid plus 100 c.c. of water. Now add, a little at a time, about 15 c.c. of 1 per cent potassium permanganate solution to destroy certain reducing materials present in the gum ghatti. The slight turbidity of the solution will disappear completely, if kept at 37° C. for a few days.

SEROLOGIC STUDIES BY THE PRECIPITATION, PRECIPITATION-FIXATION AND THE COLD FIXATION TESTS FOR SYPHILIS*

By B. S. Levine, Ph.D., Hines, Illinois

I

THE antigen precipitation reaction in the laboratory diagnosis of syphilis has earned almost universal recognition. Its appeal to the laboratory worker rests on the technical simplicity, resulting from the elimination of the hemolytic system, and in the apparent ease of performance. Its appeal to the physiologic chemist rests on the probability that the flocculation of alcoholic antigen in syphilitic serum is a direct manifestation of the union of antigen with antibody. However, since the antigen employed in the precipitation reaction is derived from the same materials and by practically the same process as the antigen used in the complement fixation procedure, no specificity of reaction bearing upon syphilis, from a theoretic consideration, can be claimed for antigen precipitation any more than for complement fixation. In either of the procedures the evaluation of the results rests on a purely empirical ground.

I have previously expressed the opinion that the phenomenon of antigen precipitation, like that of complement fixation, is controlled largely by the same conditions that control most colloidal reactions.[1] It is greatly influenced by the quantitative relationship existing between the reacting substances present in the serologic system at the time the test is performed. Such relationship varies with each serum tested.[2] It is difficult, therefore, to evolve a sufficient, yet simple system of quantitative relationships which would result in complete and proper antigen precipitation in every case.

Parallel studies in complement fixation and antigen precipitation have confirmed this statement. The truth of this assertion becomes particularly evident when the so-called combined antigen precipitation and complement-fixation tests are performed simultaneously on a large number of sera. Keining[3] made such a combined study of the Sachs-Georgi test and the Wassermann reaction and found that a positive Sachs-Georgi test occasionally yielded a negative superimposed Wassermann test, while on the other hand, a negative Sachs-Georgi might be followed by a positive superimposed Wassermann reaction. Kahn, Landau, and McDermott[4] made a similar study by combining the Kahn procedure with the complement-fixation reaction and found a disagreement of 2.0 per cent in the results.

I undertook a study of the combined complement-fixation-precipitation reaction comparing it with the cold incubation procedure, a study which had not been made previously. Some interesting and instructive results were obtained. The precipitation tests were carried out according to Kahn.[5] Upon

*From the Clinical Laboratories and Laboratory Center, Edward Hines, Jr. Hospital. Presented at Staff Conference.

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the completion of the precipitation tests, the sets were placed in the ice box and the results read the following day. No probable positive cases could be missed by such a procedure. It is believed by some[6, 7] that in the cold, antigen precipitates in some instances resulting in the introduction of nonspecfic positives. I have not found this to be the case, and therefore believe that such a procedure makes reading of the precipitates easier and more certain in the weaker reactions.

Following the period of cold storage, complement was added directly to all the tubes of the precipitation tests, and the sets incubated at 37.0° C. in the water-bath for one-half hour, the hemolytic system added, and the tubes again incubated for one-half hour. The results were then read and recorded parallel with those of the original precipitation tests. Independent complement-fixation tests were carried out on the same sera using the cold incubation method proposed by Kolmer.[8] The results were recorded parallel with the findings of the other two tests and a comparison made.

Only cases proved positive by one or more of the procedures studied were considered. Percentages of agreement or disagreement of any group of cases, therefore, bear a relation to the total number of *positive* cases assembled, and to the number of cases of each subgroup, wherever a subgroup analysis was made. Seven hundred cases were studied in this way. Of those, one hundred, or approximately 14.5 per cent were positive by one or more of the procedures used. The 100 cases were subjected to a statistical analysis, summarized below:

COMBINATION I

1. Kahn positive, Kahn-Wassermann positive	67.0%
2. Kahn negative, Kahn-Wassermann negative	7.0%
3. Kahn negative, Kahn-Wassermann positive	23.0%
4. Kahn positive, Kahn-Wassermann negative	3.0%

COMBINATION II

1. Kolmer positive, Kahn positive	67.0%
2. Kolmer positive, Kahn negative	30.0%
3. Kolmer negative, Kahn positive	3.0%

COMBINATION III

1. Kolmer positive, Kahn-Wassermann positive	91.0%
2. Kolmer positive, Kahn-Wassermann negative	6.0%
3. Kolmer positive, Kahn-Wassermann doubtful	3.0%

COMBINATION IV

1. Kolmer positive, Kahn positive, Kahn-Wassermann positive	64.0%
2. Kolmer positive, Kahn negative, Kahn-Wassermann positive	24.0%
3. Kolmer negative, Kahn positive, Kahn-Wassermann negative	3.0%
4. Kolmer positive, Kahn negative, Kahn-Wassermann negative	6.0%
5. Kolmer positive, Kahn positive, Kahn-Wassermann doubtful	3.0%

The following résumé presents the analysis more succinctly:

1. Kolmer positive, Kahn-Wassermann positive	91.0%
2. Kolmer positive, Kahn positive	67.0%
3. Kahn positive, Kahn-Wassermann positive	67.0%
4. Kolmer positive, Kahn positive, Kahn-Wassermann positive	64.0%
5. Kolmer positive, Kahn-Wassermann negative	6.0%

The above figures indicate that there is a closer agreement between the Kolmer and the Kahn-Wassermann than between the Kahn and the Kahn-Wassermann; and that the percentages of agreement between the Kahn and the Kolmer, and between the Kahn and the Kahn-Wassermann are the same, being 67.0 per cent in either case. Analysis of the subgroup of the Kahn negative and the Kahn-Wassermann positive, and of the Kahn negative and the Kolmer positive discloses that in the majority of the cases of these subgroups the intensity of the hemolytic inhibition ranged from "very strongly positive" to "moderately positive." These cases were retested by the antigen and distilled water precipitation procedure described elsewhere, for their ability to fix complement.[9] They yielded reactions of a high degree of intensity. The conclusion may be drawn from this that in the specimens under consideration the prevailing colloidal conditions were such as to inhibit the separation of the antigen-amboceptor complex. In other words, the Kahn precipitation method, like other macroscopic methods of precipitation, did not prove all-sufficient.

On the other hand, the Kahn positive and Kahn-Wassermann negative, and the Kahn positive and the Kolmer negative cases were few in number and the precipitation in such instances was weak. I hold the opinion at present that the antigen precipitation as finally worked out by Kahn offers a convenient criterion for judging the serologic status of a blood specimen, and that it apparently bids fair to replace the complement-fixation procedure in the laboratory diagnosis of syphilis. Nevertheless, the above statistical study indicates that, as things now stand, the complement-fixation procedure involving preliminary incubation in the cold may have some advantages over the macroscopic precipitation method.

It is here frankly admitted that the ultimate decision in any case depends upon the viewpoint of the syphilologist and is based upon his clinical training, observation and diagnostic judgment. It must not be forgotten that only the general mechanisms of complement fixation and of antigen precipitation are plausibly explainable on the basis of the old immunologic conception or of the interplay of physicochemical forces, such as surface tension, equilibria, etc., but the particular principles of these reactions which should spell *syphilitic specificity* are entirely unknown. Therefore, the correlation of the results of the tests with the viewpoint of one or other group of clinicians concerning the cases under study is at present the *sole* basis upon which the value of any serosyphilitic laboratory procedure is established. Such a basis is strictly empirical, and cannot be regarded as scientific. It is accepted or rejected by the clinician in accordance with its agreement or disagreement with his impressions or judgment of any case under consideration.

II

The following briefs of three histories are given as illustrations. The cases are of special interest since they represent the clinical judgment of several specialists, men of notable reputation.

CASE 1—

Laboratory Findings.—Nov. 19, 19—. Red blood cell count, 4,160,000. White blood cell count, 5,400. Hemoglobin, 69 per cent. Differential, P.M.N., 41 per cent; S.M., 48 per cent; L.M., 10 per cent; Trans., 1 per cent.

Serologic.—Wassermann positive on May 18, 19—. Bureau Wassermann 75 per cent Nov. 19, 19—. Kahn 4-plus Nov. 19, 19—. Kolmer strongly positive Nov. 19, 19—.

Physical Examination.—No scars noted on penis. The scrotal contents seem to be bound together with more or less fibrous nodules of the testicles at their bases. The left testicle in the region of the globus minor has a rather large nodule the size of a good cherry. It is hard and firm but not tender. There is evidence of several old healed sinuses of the left scrotal sac, some of which are due to attempts at aspiration, and others, according to the history, are the results of spontaneous opening and drainage. Chest: "There are a few medium fine crackling râles heard above and below the left clavicle on inspiration following expiratory cough. No other râles are heard in the chest. Whisper and spoken voice sounds show no pathologic changes." (11/20/19—.) X-ray Report: Stereoscope of Chest: "Trachea, heart, aorta, and bony framework normal. Diaphragm: Both costophrenic spaces partly obliterated. Lungs: The usual calcification and fibrosis at the hila and along all trunks. A small cloud and a few thickened trunks in each apex." (11/19/19—.) Report of Consultant in Internal Medicine: "Percussion shows dullness over both apices, particularly on the left above the clavicle and over the left apex posteriorly râles are heard after coughing. No râles were heard over the right. The temperature in this case shows a slight rise above normal. No specimens of sputum were found positive for acid-fast bacilli. The x-ray plates show distinct involvement of the left apex and slight of the right." (12/10/19—.)

Diagnosis.—Internist's: Tuberculosis, pulmonary, chronic, moderately advanced, active. G. U. Specialist's: Tuberculosis of the epididymis and seminal vesicles.

CASE 2.—Patient had sore on penis eighteen years ago; treated it himself by the use of rosin pills. States the sore healed in about two weeks. Denies any skin eruptions; afterward states his glands have never been enlarged except the right inguinal gland which was swollen at the time but declares this was due to injuring it against the corner of the table. It was incised by a physician and drained for some little time.

Electrocardiographically patient manifests: Tachycardia, simple, and somatic tremor.

Ophthalmologically patient manifests: Retinitis, low grade, secondary, bilateral; choroiditis, circ., disem., bilat.; vitreous opacities, bilat.; corneal nebulus, bilat.

Serologic Findings—

Nov. 4, 19—	Bureau Wassermann	Negative
	Kahn	Two-plus
	Kolmer	Moderately positive
Nov. 17, 19—	Bureau Wassermann	25.0 per cent
	Kahn	Three-plus
	Kolmer	Moderately positive
Nov. 30, 19—	Bureau Wassermann	50.0 per cent
	Kahn	Four-plus
	Kolmer	Strongly positive

Provocative Findings—

Dec. 5, 19—	Bureau Wassermann	Negative
	Kahn	Negative
	Kolmer	Negative
Dec. 9, 19—	Bureau Wassermann	25.0 per cent
	Kahn	Three-plus
	Kolmer	Strongly positive

Specialist in Internal Medicine Reports.—"The patient's symptoms are entirely out of proportion with what is revealed by the physical examination. I can see no good reason for the extreme dyspnea which he either suffers or affects. There is no evidence of any valvular disease of the heart. The aorta is certainly dilated, as shown in the x-ray and as indicated by the character of the second aortic sound. The possibility of myocardial disease must be seriously considered. In view of the history of syphilis, it is possible that the patient may have syphilitic disease of the heart muscle and syphilitic aortitis. However, this diagnosis is simply presumptive and by no means established. There is nothing to indicate the presence of aneurism."

Diagnosis: Dilatation of aorta. Slight enlargement of heart. Myocardial disease. Emphysema, slight.

The Syphilologist Reports.—"This patient had a sore on penis eighteen years ago for which he received only local treatment, and subsequent to it had inguinal adenitis. There is no history of treatment for syphilis in his folder. There is a conflict in the laboratory tests. The physical examination is negative for evidence of syphilis. A therapeutic test to determine the possible relation of this infection, if present, to the heart disease is recommended. Patient should be given 0.2 gm. neoarsphenamine twice a week for three weeks, and the effect on the heart noticed."

Internist's Second Report.—"The patient shows nothing in any way different from what was revealed by the examination made previous to the administration of the neoarsphenamine. He has much less dyspnea today than on the previous occasion. There can be no doubt that the patient's symptoms are largely, if not entirely, hysterical in nature. Neither the slight emphysema of the lungs, nor the heart condition could possibly cause the intense dyspnea which the patient exhibits. As far as one can judge from the examination, the treatment with neoarsphenamine has produced no change in the objective findings. I think it is impossible to come to a definite conclusion about the etiology. The evidence of syphilitic infection is certainly inconclusive. The changes in the aorta may be due to a simple arteriosclerotic process. Diagnoses remain as previously given."

CASE 3—

Laboratory Findings—

Serologic:	Nov. 10, 19—	Bureau Wassermann	25.0 per cent
		Kahn	Three-plus
		Kolmer	Mod. positive
	Nov. 19, 19—	Bureau Wassermann	Negative
		Kahn	Two-plus
		Kolmer	Weakly positive
P. S. P.	Nov. 10, 19—	First hour (intramuscular)	15.0%
		Second hour	25.0%
		Total	40.0%
	Nov. 22, 19—	First hour	20.0%
		Second hour	10.0%
		Total	30.0%
Blood Chemistry:	Nov. 10, 19—	N.P.N. 48.0 U.A. 2.2	Sugar 148
	Nov. 22, 19—	N.P.N. 40.8 U.A. 2.2	Sugar 129
Sugar Tolerance:	Nov. 26, 19—	Before sugar administration	129
		100 grams sugar administered.	
		½ hour after	175
		1 and ½ hour after	210
		2 and ½ hours after	220

First Internist Reports.—"Examinations here show the kidneys to be more or less seriously involved but to what extent it is impossible at present time to say definitely. There is an increased amount of sugar in the blood, but not enough to seriously handicap the patient at the present time. There is also some nitrogen retention present and the functional test of the kidneys is rather low. The pulse pressure is extremely low." (Nov. 26, 19—.)

First Psychiatrist Reports.—"The patient has clear insight, knows that he has heart disease and that he had difficulty in swallowing and that he had a drooling and paresis of the left face." (Nov. 26, 19—.)

Diagnosis: Facial nerve paresis, second and third branches, left, probably not central, though it may be interstitial.

Recommendation: Should be examined more extensively neurologically, after repeated Wassermann, for clearing of diagnosis in this regard.

Second Internist Reports.—"The retina shows definite arteriosclerotic changes. The sudden appearance of some paralysis of the left face, tongue, throat, and right hand suggests either a localized edema or the possibility of embolus. Apparently these symptoms have modified to some extent. These first appeared Nov. 26, 19—. This would suggest that it was probably localized edema due to the vascular spasm rather than due to embolus." (Nov. 30, 19—.)

Second Psychiatrist Reports.—"The reflexes of the upper extremities are equal and active. The knee and Achilles jerks are active and equal. Plantar response is average. There is no disturbance to light touch or vibratory sense, but the patient has some difficulty in distinguishing sharp from dull over the extremities. The patient was not tested for ataxia or the Romberg position because the least voluntary exertion on his part increased his dyspnea.

"This patient shows no frank paralysis of the muscles that are giving him discomfort. In view of his general circulatory condition, would interpret his trouble as some circulatory dysfunction involving principally the seventh, ninth, tenth, and twelfth nerves." (Nov. 30, 19—.)

Diagnosis: Paresis of left face, tongue, throat, and right hand secondary to his circulatory condition.

Third Internist Reports.—"This man presents a picture of congested heart disease. The respiration is Cheyne-Stokes variation. There is considerable edema of the feet, legs, flanks, and back. The liver is about four fingers below the costal margin. Lower border of the spleen is palpable. My impression is that there is fluid in the left pleural cavity. Rate is not much accelerated. There is no visible pulsation in the neck. Cardiac impulse is very faint against the hand. Percussion over the heart shows dullness as follows: M.R. 2d, 5 cm.; 3d, 6 cm.; 4th, 7 cm.; 5th, 8 cm.; M.L. 2d, 6 cm.; 3d, 9 cm.; 4th, 10½ cm.; 5th, 15 cm.; 6th, 17½ cm. Shift in right 4th, from left lateral to right lateral posture is 6 cm.

"On auscultation no murmurs are audible. There is not much alteration in the second sound. The sounds of the heart are rather weak, especially in the recumbent posture, though leaning forward cardiac impulse is not strongly felt against the hand, nor is its intensity considerable to the ear." (Dec. 6, 19—.)

Diagnoses: Congestive heart disease associated with arteriosclerosis. Pericarditis with effusion.

Fourth Internist Reports.—"The physical examination, as well as the x-ray films, indicates a marked enlargement of the heart and the absence of any evidence of obliteration of the cardiohepatic effusion, especially so since there is no pulsus paradoxus and no change in the intensity of the heart sounds with change of position by the patient.

"There is present today a slight edema of the left ankle which has not been present up to now, and it is noteworthy that a few râles can be heard at both bases behind.

"The high blood sugar with fasting stomach, together with the rapid rise in blood sugar following ingestion of 100 gm. glucose and the delayed return to normal indicates a definite diabetes. The constant presence of albumin, at times in large amounts, with the presence of casts, together with a high nitrogen blood content and the evidence of retinal hemorrhage and exudate, indicates a definite nephritis.

"The high diastolic pressure when considered with the retinal arteriosclerosis and the evidence of nephritis, could indicate a generalized arteriosclerosis absolutely abnormal for a man of his age." (Dec. 13, 19—.)

Diagnoses: Myocarditis, with hypertrophy, and dilatation of the heart; etiologic factor not found. Arteriosclerosis, general. Nephritis, interstitial, chronic. Diabetes, mellitus.

Syphilologist Reports.—"There is nothing in the history to indicate syphilitic infection. The physical examination is negative for syphilis except the cranial nerve involvement. Definite diagnosis should await spinal puncture." (Dec. 14, 19—.) (Patient refused to submit to spinal puncture.)

III

General theoretic considerations and the impressions gained from numerous histories, of which the three cited are offered as examples, lead to the conclusion that to speak of one seroluetic laboratory procedure as being per se more specific than another is scientific folly. However, the cold incubation and the Kahn precipitation procedures belong to the type of immunologic

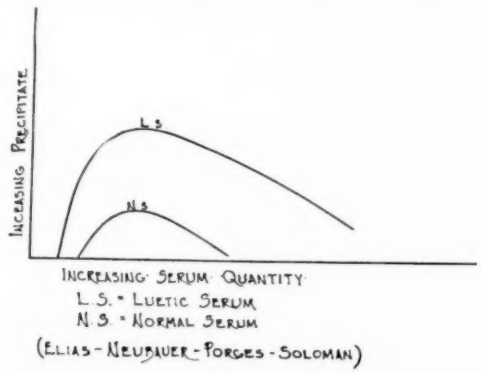

Fig. 1.

tests which the laboratorian conveniently calls "very sensitive." It was on this basis only that the two procedures were studied comparatively.

Porges and Neubauer[10] demonstrated that lecithin and cholesterol are precipitated by a maximum of another colloid. An electric neutralization takes place between the oppositely charged colloids, and the precipitate occurs when the colloids are in proper balance. However, when one of the colloids is in excess of the proper balance, it exerts a protective influence upon the "reaction product." The reversible influence is proportional to the amount in excess. A point is finally reached where the precipitate completely disappears. They proved further that lecithin and cholesterol are anodic and react in the optimum quantitative relationship with kathodic colloids so as to form a precipitate.

These authors in collaboration with Elias and Solomon[11] confirmed the fact that the hydrophylic colloid lecithin is electronegative. And further, that in its precipitation under the influence of the luetic serum it followed a characteristic curve. The curve starts with zero, increases with the volume of the serum added, reaches a maximum and thereafter declines to zero. Nor-

mal serum also precipitates lecithin, but the zone of precipitation is smaller and the reaction time longer. This is graphically shown in the following diagram.

Cholesterol augments the curve of precipitation. Hence the effectiveness of the Kahn antigen in the majority of sera tested. Exceptions, however, occur for the following reasons: First, the electropositive constituents of the many sera are normally variable; second, the degree of positiveness of the sera is of wide range; third, in the Kahn test the antigen is used in three fixed quantities. It is theoretically conceivable, therefore, that in some positive cases the optimum quantitative relationship between the oppositely charged reacting colloids is not obtained by the Kahn test. This may occur in any one or all of the ratios of the Kahn antigen, thus yielding doubtful or false results.

The following table is of interest in this connection.

SERUM NO.	1	2	3	4	5	6	7	8	9	10
Kahn	2.1.4	4.4.4	4.4.4	4.4.4	0.4.4	1.2.4	4.4.0	0.1.3	0.0.3	0.0.3
K.-Wass.	2.4.2	2.0.4	3.3.1	4.3.2	1.2.2	4.4.4	4.4.4	4.3.2	4.3.3	4.4.3

It will be observed that in some instances the Kahn was weak or negative while the Kahn-Wassermann was strongly positive, and vice versa. The table also indicates that averaging the readings of the visible precipitates in the three tubes used in the Kahn method in order to deduce an expression of the actual condition in the serum tested appears to be a mistaken procedure.

SUMMARY AND CONCLUSIONS

Seven hundred sera were tested for the presence of antigen combining antibody by the Kahn, Kahn-Wassermann combined, and the Kolmer procedures. One hundred sera, or about 14.5 per cent were positive by one or more of the methods used. The positive values were subjected to statistico-analytic study.

It was pointed out that from a theoretic viewpoint no syphilitic specificity can be claimed for any of the laboratory tests for syphilis now in use.

Excerpts of histories are presented to show instances in which the Kahn and the Kolmer tests gave positive results, yet, the final diagnosis made no reference to syphilis.

The following conclusions are drawn:

1. The results obtained by the cold incubation and the Kahn-Wassermann fixation procedures closely agree.

2. The percentage agreement between the Kahn and Kahn-Wassermann, and the Kahn and Kolmer are identical (67.0 per cent).

3. The Kahn yields negative results in about 25.0 per cent of the cases where antigen combining substance is present. The Kolmer and Kahn-Wassermann differed in only 5.0 per cent of the cases.

4. Averaging the three readings of the Kahn precipitates to obtain an interpretation of the results is a mistaken procedure.

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A COMPARATIVE STUDY OF 2000 HINTON, KAHN AND WASSERMANN TESTS*

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IN 1927, Dr. W. A. Hinton[1] of the Wassermann Laboratory of the Massachusetts Department of Public Health described a glycerol-cholesterol precipitation test for syphilis, which has become known as the Hinton test. At this time, he compared the results obtained with his test and the Wassermann test in about 500 consecutive cases, syphilitic and nonsyphilitic. In his opinion, the Hinton test was simple, easily read and sensitive. It apparently possessed greater clinical value than the Wassermann test, employed in his laboratory,[2] and required less labor, apparatus, and materials. In view of these favorable results, the Hinton test has been employed in our laboratory, during the past year, in parallel with the Wassermann and Kahn tests, which are in routine use here. This paper records the results of that study.

At a symposium on syphilis held at the Boston Dispensary in December, 1927, favorable reports on the sensitiveness and specificity of the Hinton test were given. It was also found to give a larger number of positive reactions in treated cases than the Hinton Wassermann test.[3, 4, 5]

Smith[6] and Cheever and Splaine,[3] reporting on the Boston Dispensary group of 1610 cases with 2026 serums, found 71.7 per cent agreement between the three tests (1444 serums). Smith[6] and Munter[7] reporting on the Peter Bent Brigham Hospital group of 2120 cases with 2331 serums, examined by the Wassermann and Hinton tests, found 90.6 per cent agreement between the tests. In the Massachusetts General Hospital group, Smith[6] found 80.2 per cent agreement between the Wassermann and Hinton tests in 1759 serums. Smith also reported that in 176 cases from private practice, there was 65.9 per cent agreement between the three tests; there was 74.43 per cent agreement between the Hinton and Kahn tests in this series. In this group there were 17.61 per cent Wassermann positive, 40.34 per cent Hinton positive, and 24.43 per cent Kahn positive reactions; the small percentage of agreement between the three tests was due largely to the great difference in sensitiveness between the Hinton and Wassermann tests. Smith also reported on other hospital groups. The Hinton-Wassermann technic was used by these workers. Smith concluded that as a rule the Hinton test gave a smaller number of so-called doubtful readings, the incidence of false positives was reduced, and it was claimed that the reaction became positive earlier than the Wassermann, and at least as early as the Kahn.

Ferguson and Greenfield,[8] in examining 460 serums by the three tests found complete agreement in 88.5 per cent, 92.4 per cent agreement between the

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Hinton and Kahn tests, 93 per cent agreement between the Kahn and Wassermann tests, and 91.3 per cent between the Hinton and Wassermann tests. These results were not correlated with clinical data. They concluded that the Hinton test was economical, simple and highly sensitive for syphilitic serums, and that there were indications that the test was somewhat more sensitive and reliable than the Kahn test. They did not think that it was comparable in reliability with a carefully standardized Wassermann technic (they used Medical Research Committee's No. 4 method), but it appeared to pick out certain types of cases, especially "treated" cases, and "latent" cases, where the Wassermann test at times failed. The Hinton test must be reserved as a supplementary test to the Wassermann.

Osmond[9] found 91.6 per cent agreement between the Wassermann and Hinton tests in 500 serum tests. On 201 other serums examined by the Kahn and Hinton tests, 93 per cent agreement was found; of the 14 disagreeing reactions, 7 were in favor of the Kahn test, and 7 in favor of the Hinton. He concluded that the Hinton test could claim to be the equal, at least, of the Wassermann and Kahn tests. It was easier to perform and to read than the Kahn test.

Jones[13] examined the serums from 400 women from a prenatal clinic; 384 serums were negative by both tests, and 7 were positive by both tests, giving 97.75 per cent agreement. Of the remaining 9 serums, there were 3 which were Hinton positive-Wassermann negative; one was from a treated case of syphilis; one was considered positive from the history and physical signs; the third was considered nonsyphilitic. Of the other 6, which were Hinton negative, 5 gave ± reactions with the cholesterinized antigen only; the sixth gave a 2+ reaction; all were considered nonspecific. Jones concluded that a routine blood Wassermann test was of definite value on prenatal patients in the diagnosis of syphilis; the value was increased if checked by a precipitation test, the best of which he had found to be the Hinton test.

Todd[15] examined 728 serums, finding 96.84 per cent agreement between the Kolmer-Wassermann test and the Hinton test. He concluded that the Hinton test made a superior check test for the complement-fixation test, or for one of the other precipitation-flocculation tests; and that, if the complement-fixation test was to be discarded, the Kline antigen in the Kahn tube test, checked by the Hinton test, made a promisingly satisfactory serologic examination for syphilis.

TESTS IN ROCHESTER HEALTH BUREAU LABORATORIES

The tests included in this paper were carried out between August, 1928, and September, 1929, and consisted of two series of 1000 serums each. In the first series serum specimens of sufficient quantity, after the routine Wassermann and Kahn tests were completed, were selected. Some of these were positive and some negative. In the second series, serums which gave positive reactions by either the Wassermann or Kahn test or by both, as well as serums from treated cases, negative by both tests, were selected. A few routine negatives were included each time tests were set up, as controls. The tests were all carried out with antigen obtained from Dr. Hinton. Between

the two series of tests, the writer, through the courtesy of Dr. Hinton, visited the Wassermann Laboratory of the Massachusetts Department of Public Health and the laboratory of the Boston Dispensary in order to observe the setting up and reading of tests.

Following is the technic of the Hinton test.[1, 10] These reagents are required:

1. Three per cent and 5 per cent sodium chloride solutions.

2. A 50 per cent solution of glycerol in distilled water, using redistilled neutral glycerin.

3. The stock indicator, which was obtained from Dr. Hinton, is an ether-insoluble, alcohol-soluble extract of beef muscle made from round beefsteak. The meat is freed from fat and connective tissue, finely ground, and dried for four to five days at 56° C. in a large flat dish. The powdered tissue is then extracted 4 or more times with ether (U.S.P.) in a glass-stoppered bottle, one gram of the powder to 4 c.c. of ether for each extraction. This process is completed when the last extract is colorless. Finally, all the ether is poured off and the residue is dried on filter paper, after which it is extracted with 95 per cent alcohol in a glass-stoppered bottle for three days at room temperature, 1 part is added to 9 parts of 0.7 per cent cholesterol (Merck) in absolute alcohol. It is then allowed to stand for four to eight weeks to ripen and is then titrated and adjusted. It appears to keep indefinitely.

The serums to be used were heated for one-half hour at 55° C., as for the Kahn and Wassermann tests. Three tubes were used, containing 0.1 c.c., 0.3 c.c., and 0.5 c.c. respectively of serum.

Glycerinated indicator was prepared freshly for each day's work. To 1 c.c. of stock indicator was added 2 c.c. of 5 per cent salt solution; it was shaken vigorously; then 12 c.c. more of salt solution were added, followed by shaking; then 15 c.c. of glycerol solution were added, and the mixture was well shaken. The glycerinated indicator from the 3 per cent salt solution was made in the same way. The latter was added in 0.5 c.c. amounts to the tubes containing 0.1 c.c. of serum, while the tubes containing 0.3 c.c. and 0.5 c.c. of serum received the 5 per cent salt solution glycerinated indicator. (In our first series, we used both salt solution indicators, but in the second series only the 5 per cent, as Dr. Hinton had found by experience that this gave better results.)

The racks of tubes were then well shaken, incubated overnight (sixteen hours) in a 37° C. water-bath, taken out in the morning and read within an hour.

Negative tests show a uniformly distributed turbidity, or even a clearing, but no sizable agglutinated masses. Weak or moderate reactions show a water clear fluid, throughout which may be suspended many small, or a few large clumps of agglutinated particles of cholesterol (recorded "W" or "M"). Strong reactions show from one to several large clumps near the surface of the fluid (recorded "+"). When two or more tubes show a weak reaction, the result is recorded as doubtful, while one or more "M" or "+" is recorded as positive. For our tables, these doubtful reactions have been designated as "P" (partial), and the positive reactions as "M" (marked).

All tests were read by two workers, and recorded, before the case history and the results of the Wassermann and Kahn tests were consulted.

The Kahn antigen used in this laboratory is purchased from the Michigan Department of Health. A two-tube test is used, omitting the tube containing the largest amount of antigen dilution (0.05 c.c.). This is done in order to secure uniformity, as many serums are not sufficient in quantity for a three-tube test, otherwise the test follows the usual Kahn technic.[11] About 100 tests are done daily and all tests are read by two workers.

The technic used in the Wassermann test is similar to that used in the New York State Laboratory at Albany. The total volume of the test is 0.5 c.c., i.e., one-tenth that of the original Wassermann. All reagents are diluted in accordance with preliminary titrations so that 0.1 c.c. may be pipetted in the test. The complement is the pooled serum of at least 6 guinea pigs, each serum having been previously tested for hemolytic activity, natural antisheep amboceptor, and nonspecific fixability with the cholesterinized antigen. The complement is titrated daily with 5 per cent washed sheep cells sensitized with 2 units of amboceptor, and 2 units of complement are used in the test. Each specimen of serum is tested with two antigens, an alcoholic extract of beef heart reinforced with 0.4 per cent cholesterin, and an acetone-insoluble extract of beef heart prepared according to Bordet's method. Amboceptor and antigens are supplied by the New York State Laboratory. The tests with both antigens are kept for four hours at 3° to 6° C. for the period of fixation. Sensitized sheep cells are then added, and the tests incubated in the water-bath at 37° C. for fifteen minutes (until controls with serum alone, and antigen alone, show complete hemolysis). Readings are then made, using a color standard, all tubes showing 2+ or more being centrifugalized, and the percentage of inhibition of hemolysis read from the supernatant fluid. About 150 serums are tested daily. Certain serums from cases with histories suggestive of early syphilis, and others which gave a reaction with the Kahn test were set up with the cholesterinized antigen only, and were given one-half hour fixation in the 37° C. water-bath in addition to the ice box fixation. Of this number, only 8 specimens gave reactions with the water-bath fixation at 37° C., and these were confirmed by the Kahn test, or the Hinton test, or by both. These specimens are indicated in Table VII.

PRESENTATION OF RESULTS

The serums included in the tests presented in this paper came from two main sources: (1) the hospitals of the city which include three venereal disease clinics, and a city venereal disease clinic, and (2) physicians in the city. From the hospital and venereal disease clinic patients, fairly sufficient and accurate histories can be obtained. Some of the history blanks, however, accompanying specimens sent in by physicians give very little information concerning the patient while others may be completely filled out.

From the data available, the 2000 serums have been divided into three classes: (1) those from treated cases of syphilis; (2) those from cases which have been diagnosed as syphilis but in which no history of treatment is given, and those from cases, particularly early ones, in which history or symptoms suggest syphilis; and (3) those called routine, either because no history was given, or because the history did not suggest syphilis. (Table VII.)

It is evident that if more information were available some of the serums classified as untreated, suggestive, and routine might have been included in a different group. The serums in the treated case group were accurately known and the findings of the three tests in this group are most valuable. It is in this treated case group that the Hinton and Kahn tests are claimed to be more sensitive than the Wassermann test.

For the tables which are presented, the following key has been used: Wassermann reactions 2+, 3+, 4+; Kahn reactions 2+, 3+, 4+; and Hinton reactions M and +, have been designated marked (M). Wassermann reactions ± and +, Kahn reactions ± and +, and Hinton ± have been designated partial (P). Negative results have been designated negative (N). The Wassermann, Kahn, and Hinton tests have been called W, K, and H respectively.

Tables I and II show the results of the three tests on the first 1000 serums examined, where there was a large percentage of serums negative by all three tests.

TABLE I

RESULTS OF FIRST 1000 WASSERMANN, KAHN, AND HINTON TESTS. SERUMS SELECTED ON BASIS OF QUANTITY. LARGE PREPONDERANCE OF NEGATIVE REACTIONS

	WASSERMANN			KAHN			HINTON		
	M	P	N	M	P	N	M	P	N
	125			125			120	0	5
	6				6		2	0	4
	17					17	10	0	7
		1		1			1	0	0
		10			10		7	0	3
		19				19	3	0	16
			3	3			3	0	0
			1		1		0	1	0
			818			818	5	0	813
Totals	148	30	822	129	17	854	151	1	848

TABLE II

PERCENTAGES OF AGREEMENT BETWEEN RESULTS OF FIRST 1000 WASSERMANN, KAHN, AND HINTON TESTS

Agreements	W AND K	AND H	W AND K	W AND H	K AND H
Marked reactions (M)	122	121 130	131	126 132	143 124	133
Partial reactions (P)	8	9 0	11	16 11	0 10	1
Negative	813	813 813	818	818 813	813 836	836
Total Agreements	943	(94.3%)	960	(96.0%)	956	(95.6%)	970	(97.0%)
Disagreements								
W: M 17 and P 19, K neg.			36					
K: M 3 and P 1, W neg.			4					
W: M 16 and P 19, H neg.				35				
H: M 8 and P 1, W neg				9				
K: M 5 and P 7, H neg.						12		
H: M 18 and P 0, K neg.						18		
Total Disagreements	57	(5.7%)	40	(4.0%)	44	(4.4%)	30	(3.0%)
Total Tests	1000	(100.0%)	1000	(100.0%)	1000	(100.0%)	1000	(100.0%)

Tables III and IV show the results on the second 1000 serums, where a large number of positive serums and serums from treated cases of syphilis were included.

The comparison of the results set forth in these tables demonstrates clearly the effect of selection of specimens upon the statistics of agreement between tests. With tests which give very few false positive results, a series

TABLE III

RESULTS OF SECOND 1000 WASSERMANN, KAHN, AND HINTON TESTS. SERUMS SELECTED ON BASIS OF HISTORY OF SYPHILIS AND POSITIVE WASSERMANN OR KAHN REACTIONS

	WASSERMANN			KAHN			HINTON		
	M	P	N	M	P	N	M	P	N
	433			433			283	111	39
	25				25		7	11	7
	64					64	1	26	37
		24		24			3	10	11
		9			9		0	3	6
		42				42	0	10	32
			4	4			0	1	3
			2		2		0	0	2
			397			397	3	18	376
Totals	522	75	403	461	36	503	297	190	513

TABLE IV

PERCENTAGES OF AGREEMENT BETWEEN RESULTS OF SECOND 1000 WASSERMANN, KAHN, AND HINTON TESTS

Agreements	W AND K AND H			W AND K		W AND H		K AND H	
Marked reactions (M)	412	407	293	458	457	439	285	408	303
Partial reactions (P)	16	21	135	33	34	26	180	21	126
Negative	376	376	376	397	397	381	381	445	445
Total Agreements	804		(80.4%)	888	(88.8%)	846	(84.6%)	874	(87.4%)
Disagreements									
W: M 64 and P 42. K neg.				106					
K: M 4 and P 2. W neg.				6					
W: M 83 and P 49. H neg.						132			
H: M 3 and P 19. W neg.						22			
K: M 53 and P 15. H neg.								68	
H: M 3 and P 55. K neg.								58	
Total Disagreements	196		(19.6%)	112	(11.2%)	154	(15.4%)	126	(12.6%)
Total Tests	1000		(100.0%)	1000	(100.0%)	1000	(100.0%)	1000	(100.0%)

comprised largely of specimens giving negative reactions will show a high percentage of agreement among the tests. On the other hand, a series composed of specimens selected on the basis of a special origin, in this case from syphilities, or because of their special reactions with one or more tests, will bring out most clearly the differing capacities of tests to indicate positive results. In comparing the results obtained in the first series of 1000 unselected specimens with those in the second 1000 selected specimens, it is seen that the agreement between the three tests dropped from 94.3 per cent

to 80.4 per cent, the agreement between the Wassermann and Kahn tests from 96.0 per cent to 88.8 per cent, the agreement between the Wassermann and Hinton tests from 95.6 to 84.6 per cent, and the agreement between the Kahn and Hinton tests from 97.0 per cent to 87.4 per cent.

When the two series are combined, the percentages of agreement between the tests fall into an intermediate position, as shown in Tables V and VI. The agreement among the three tests in 2000 cases was 87.35 per cent, between the Wassermann and Kahn tests 92.4 per cent, between the Wassermann and Hinton tests 90.1 per cent and between the Kahn and Hinton tests 92.2 per cent.

TABLE V

RESULTS OF 2000 WASSERMANN, KAHN, AND HINTON TESTS

	WASSERMANN			KAHN			HINTON		
	M	P	N	M	P	N	M	P	N
	558			558			403	111	44
	31				31		9	11	11
	81					81	11	26	44
		25		25			4	10	11
		19			19		7	3	9
		61				61	3	10	48
			7	7			3	1	3
			3		3		0	1	2
			1215			1215	8	18	1189
Totals	670	105	1225	590	53	1357	448	191	1361

TABLE VI

PERCENTAGES OF AGREEMENT BETWEEN RESULTS OF 2000 WASSERMANN, HINTON, AND KAHN TESTS

Agreements	W AND K AND H			W AND K		W AND H		K AND H	
Marked reactions (M)	534	528	423	589	583	571	428	532	436
Partial reactions (P)	24	30	135	44	50	37	180	31	127
Negative	1189	1189	1189	1215	1215	1194	1194	1281	1281
Total Agreements	1747	(87.35%)		1848	(92.4%)	1802	(90.1%)	1844	(92.2%)
Disagreements									
W: M 81 and P 61. K neg.				142					
K: M 7 and P 3. W neg.				10					
W: M 99 and P 68. H neg.						167			
H: M 11 and P 20. W neg.						31			
K: M 58 and P 22. H neg.								80	
H: M 21 and P 55. K neg.								76	
Total Disagreements	253	(12.65%)		152	(7.6%)	198	(9.9%)	156	(7.8%)
Total Tests	2000	(100.00%)		2000	(100.0%)	2000	(100.0%)	2000	(100.0%)

Table VI shows that the agreement between the Wassermann and Kahn tests, and between the Kahn and Hinton tests, is practically the same, while that between the Wassermann and Hinton tests is slightly lower. There are 156 disagreements between the Kahn and Hinton tests, 80 being marked or partial Kahn tests, and 76 marked or partial Hinton tests. Of the 152 disagreements between the Wassermann and Kahn tests, 142 gave marked or

partial reactions by the Wassermann test, and of that 142, 110 were from treated cases of syphilis The Kahn test gave 7 marked and 3 partial reactions, whereas the Wassermann test was negative. Five of these serums were from treated cases of syphilis. Of the 198 disagreements between the Wassermann and Hinton tests, 167 gave marked or partial reactions by the Wassermann test; of these 167, 122, were from treated cases. Of the 31 serums, giving marked or partial reactions with the Hinton test, and no reaction with the Wassermann test, 25 were from treated cases of syphilis.

TABLE VII

CORRELATION OF RESULTS WITH HISTORIES IN 2000 CASES

660 Treated Cases, 33.0%

	W.		H.		K.	
Marked	414	62.7%	259	39.2%	342	51.8%
Partial	71	10.8%	129	19.6%	40	6.1%
Negative	175	26.5%	272	41.2%	278	42.1%
	660	100.0%	660	100.0%	660	100.0%

194 Untreated Cases and Cases With Suggestive Histories, 9.7%

	W.		H.		K.	
Marked	*105	54.1%	79	40.7%	97	50.0%
Partial	9	4.6%	24	12.4%	4	2.1%
Negative	80	41.3%	91	46.9%	93	47.9%
	194	100.0%	194	100.0%	194	100.0%

1146 Routine Cases, 57.3%

	W.		H.		K.	
Marked	°151	13.2%	110	9.6%	151	13.2%
Partial	›25	2.2%	38	3.3%	9	0.8%
Negative	970	84.6%	998	87.1%	986	86.0%
	1146	100.0%	1146	100.0%	1146	100.0%

*One specimen
°One specimen
›Six specimens
} These specimens gave reactions with the Wassermann test, with fixation at 37° C. for thirty minutes, but not with four hours at 3° to 6° C.

Table VII shows the correlation of results with histories. It shows that approximately the same number of reactions in treated cases of syphilis were obtained by the Kahn and Hinton tests. The Hinton test gave a much larger number of partial reactions. This may be partly accounted for by the fact that the final result of the readings of the two-tube Kahn test is higher than with the three-tube Kahn test, because the least sensitive tube is omitted. Both tests were far behind the Wassermann test in the number of marked or partial reactions obtained.

These results on treated cases of syphilis were not unexpected, though not in agreement with most published reports of the Kahn and Hinton tests. The difference lies, probably, in the Wassermann technic used. The New York State Laboratory method, which is employed in this laboratory, is a sensitive one; far more so than that used by Dr. Hinton and his associates. This is clearly shown in the report of Gilbert and Langworthy,[12] in which in 1926 seven laboratories conducted tests on 252 serums, each laboratory using

its own Wassermann technic. Among the laboratories were those of Dr. Hinton, and the New York State Laboratory at Albany. The Albany laboratory gave 147 marked and partial reactions (with no reactions in control cases) while the Boston laboratory gave only 41. In that same series of comparative tests, Dr. Kahn performed his precipitation test on the 252 serums, obtaining only 110 marked and partial reactions, as against Albany's 147 marked and partial reactions with the Wassermann test.

The results shown in Table VII in treated cases, confirm this work. Austin and Frey[14] found that in 393 serums from treated cases of syphilis, the Wassermann test (New York State Laboratory method) was somewhat more sensitive than the Kahn test, giving 89.1 per cent of marked or partial reactions, while the Kahn test gave 86.1 per cent.

TABLE VIII

ANALYSIS OF DISAGREEMENTS IN 253 CASES

DISAGREEMENTS	WASSERMANN			HINTON			KAHN			TYPE OF CASE
	M	P	N	M	P	N	M	P	N	
W (M or P) KH (N)	39	37	0	0	0	76	0	0	76	
W (N) KH (M or P)	0	0	2	0	2	0	1	1	0	Treated
K (M or P) WH (N)	0	0	5	0	0	5	3	2	0	Cases
K (N) WH (M or P)	24	10	0	6	28	0	0	0	34	of
H (M or P) WK (N)	0	0	23	6	17	0	0	0	23	Syphilis
H (N) WK (M or P)	36	10	0	0	0	46	30	16	0	
Total	99	57	30	12	47	127	34	19	133	186
W (M or P) KH (N)	1	2	0	0	0	3	0	0	3	
W (N) KH (M or P)	0	0	0	0	0	0	0	0	0	Cases
K (M or P) WH (N)	0	0	0	0	0	0	0	0	0	With
K (N) WH (M or P)	9	1	0	3	7	0	0	0	10	Suggestive
H (M or P) WK (N)	0	0	1	1	0	0	0	0	1	Histories
H (N) WK (M or P)	6	3	0	0	0	9	8	1	0	
Total	16	6	1	4	7	12	8	1	14	23
W (M or P) KH (N)	4	9	0	0	0	13	0	0	13	
W (N) KH (M or P)	0	0	3	3	0	0	3	0	0	
K (M or P) WH (N)	0	0	0	0	0	0	0	0	0	Routine
K (N) WH (M or P)	4	2	0	5	1	0	0	0	6	Cases
H (M or P) WK (N)	0	0	2	1	1	0	0	0	2	
H (N) WK (M or P)	13	7	0	0	0	20	17	3	0	
Total	21	18	5	9	2	33	20	3	21	44
Grand Total	136	81	36	25	56	172	62	23	168	253

Table VI shows that there was disagreement between the three tests in 253 of the 2000 serums. The records of the large number of treated cases included in Table VIII give it an especial interest as more numerous disagreements between the results of the various tests are to be expected in this group.

If the Wassermann test only had been used, 30 possible marked and partial reactions in treated cases of syphilis would have been missed; if the Hinton test only had been used, 127 possible marked and partial reactions would have been missed; if the Kahn test only had been used, 133 possible marked and partial reactions would have been missed. The Hinton test is slightly

more sensitive than the Kahn test; while both are definitely inferior to the Wassermann test in the number of reactions obtained.

CASES GIVING POSITIVE REACTION WITH ONLY ONE OF THE TESTS

It seemed worth while to analyze here the routine, and suggestive cases, whose serums gave a marked or partial reaction with only one of the three tests.

There were no positive Kahn reactions with serums from cases in these two groups.

The Hinton test gave reactions in three such cases as follows:

CASE 1.—No. 20713, J. M., male, aged forty-nine, single, laborer. Serum tested Sept. 28, 1928. Wassermann and Kahn tests were negative, Hinton test positive. Spinal fluid No. 20794 gave a negative Wassermann reaction, and a colloidal gold reading 5555553332. Patient died Sept. 30, 1928. The autopsy revealed malignant bacterial endocarditis, purulent meningitis, old pulmonary tuberculosis with a bean-sized cavity in right lung, fractured skull, and subdural clot. Blood cultures revealed hemolytic streptococci. This case is of interest because Dr. Hinton told me that bacterial endocarditis is a disease in which a false positive Hinton reaction may be obtained. This reaction may be regarded as nonspecific.

CASE 2.—No. 16491, J. G., aged sixty, laborer, with 4 children. Tested July 7, 1929. The Wassermann and Kahn tests were negative, the Hinton test positive. The diagnosis given was question of arthritis. Two specimens of blood from this patient, examined in March, 1928, gave no reaction with the Kahn and Wassermann tests.

CASE 3.—No. 22373, Mrs. C. B., aged forty-eight, housewife, married, with 2 children. Tested Oct. 19, 1928. The Wassermann and Kahn tests were negative, the Hinton test positive. The diagnosis was given as rash. No more specimens were received for examination and a letter sent to the physician concerning the patient was not answered.

From the information at hand, it is not possible to say whether or not these two Hinton reactions (Nos. 2 and 3) are specific.

The Wassermann test gave sixteen reactions, unsupported by the Kahn and Hinton tests. These were from patients in the routine and suggestive history groups:

CASE 1.—No. 23420, Mrs. C. B., aged thirty-six, with 8 children. Serum tested Nov. 1, 1928, gave a ± reaction with the Bordet antigen only, while the cholesterinized antigen, the Kahn and Hinton tests were negative. No more specimens were received, and no reply was received from the letter sent to the physician. This specimen was sent in for routine examination.

CASE 2.—No. 22914, Mrs. J. D., aged forty-seven, widowed, with no children. Serum tested Oct. 26, 1929, gave a + reaction with both Bordet and cholesterinized antigens, while the Kahn and Hinton tests were negative. The diagnosis was abdominal tumor. No more specimens were received, and there was no reply to a letter sent to the physician.

CASE 3.—No. 21980, Mr. C. H., aged twenty-seven, laborer, single. Serum tested Oct. 15, 1929, gave a ± reaction with the cholesterinized antigen only, while the Kahn and Hinton tests were negative. The diagnosis was ulcer of the lip. No more specimens were received. In answer to the letter, the physician said that he regarded the reaction as nonspecific.

CASE 4.—No. 15293, Mr. P. L. Serum tested June 19, 1929, gave a 2+ reaction with the Bordet antigen only, while the cholesterinized antigen, Kahn and Hinton tests were negative. No more specimens were received and physician did not recall patient on Oct. 11, 1929.

CASE 5.—No. 19409, Mr. J. T., aged twenty-nine, stone cutter, married, with 1 child. Serum tested Aug. 6, 1929, gave a ± reaction with the cholesterinized antigen while the Kahn and Hinton tests were negative. Two specimens of blood from this patient were examined at the State Laboratory in Albany, one in 1921, and one in 1922, and each gave a 4+ Wassermann reaction. Two specimens of spinal fluid examined here in 1929 gave 4+ reactions; 4 specimens of blood gave 4+ reactions; 3 of these specimens of blood gave positive Kahn reactions also. The patient's wife's serum gave a 4+ reaction while the child was negative. The patient is now in the Rochester State Hospital for the Insane, where he is considered to be syphilitic.

CASE 6.—No. 23189, Mrs. C. P., aged thirty-eight, married, no children. Serum tested Oct. 30, 1928, gave a + reaction with both antigens, while the Kahn and Hinton tests were negative. She had been admitted to the Rochester General Hospital three months after abortion with apparent left tuboovarian abscess and pelvic abscess. She had a high temperature which disappeared after the removal of left tuboovarian abscess and drainage of the pelvic abscess. She was discharged Jan. 14, 1929, with condition improved.

CASE 7.—No. 23462, Mr. C. W., aged seventy, married. Serum tested Nov. 2, 1928, gave a + reaction with the cholesterinized antigen only, while the Kahn and Hinton tests were negative. The patient had enlarged prostate, arteriosclerosis and phlebitis with swelling and edema of the feet. The physician wrote that the symptoms were not suggestive of syphilis; the patient did not return to his care, and a second specimen was not sent in.

CASE 8.—No. 16627, Mr. J. B., aged forty, chauffeur, single. Serum tested July 3, 1929, gave a ± reaction with the cholesterinized antigen, while the Kahn and Hinton tests were negative. There were two recent previous specimens: No. 15229 (June 17, 1929) gave ± with the Bordet antigen, and 3+ with the cholesterinized antigen; No. 16131 (June 27, 1929) was negative with both antigens. Both these specimens had negative Kahn tests. The patient was referred from the Rochester General Hospital to the Veterans' Bureau, and is not in the city now.

CASE 9.—No. 16022, Mr. R. I., aged thirty-four, colored, single. Serum tested June 27, 1929, gave a + reaction with the cholesterinized antigen only, while the Kahn and Hinton tests were negative. A specimen a year previous, No. 13484 (June 18, 1928), gave no reaction with the Wassermann and Kahn tests. A specimen examined at the State Laboratory in Albany, No. 29971, gave no reaction with the Wassermann test. The patient is now in the Iola Tuberculosis Sanatorium.

CASE 10.—No. 23194, Miss E. H., aged thirty-two, single. Serum tested Oct. 30, 1928, gave a + reaction with the cholesterinized antigen only, while the Kahn and Hinton tests were negative. The diagnosis was carcinoma of the cervix. A second specimen, No. 23777 (Nov. 7, 1928), gave no reaction with the Kahn and Wassermann tests.

CASE 11.—No. 22273, Mr. A. V., aged forty-seven, fireman, married with one child. Serum tested Oct. 18, 1928, gave a ± reaction with the Bordet antigen, and a + reaction with the cholesterinized antigen, while the Kahn and Hinton tests were negative. The diagnosis was psychoneurosis, hypochondria, and question of duodenal ulcer. A second blood specimen No. 23748 (Nov. 6, 1928) and a spinal fluid No. 23634 (Sept. 24, 1929) gave no reactions with the Kahn and Wassermann tests, and the colloidal gold was negative.

CASE 12.—No. 23821, Mrs. E. P., aged fifty, no children, worker in cafeteria. Serum tested Nov. 8, 1928, gave a 2+ reaction with the cholesterinized antigen, while the Kahn and Hinton tests were negative. The diagnosis was persistent ulcer of the leg. A second specimen No. 24353 (Nov. 15, 1928) gave a ± reaction both with the cholesterinized antigen and with the Kahn test. The patient had been referred to the physician in connection with compensation; the condition was not regarded as syphilitic.

CASE 13.—No. 11913, Mrs. E. F., aged forty-five, housewife, one child. Serum tested May 7, 1929, gave a 3+ reaction with cholesterinized antigen only, while the Kahn and

Hinton tests were negative. The diagnosis was backache in a woman of forty-five with one healthy child three or four years of age. The previous specimens No. 9345 (April 10, 1929) gave a 3+ reaction with the cholesterinized antigen, and a negative Kahn test; and No. 10459 (April 22, 1929) gave a 4+ reaction with the cholesterinized antigen, and a negative Kahn test. A specimen tested at the State Laboratory, No. 19351 (May 23, 1929) gave a ± reaction with the cholesterinized antigen only. The physician said there was no clinical evidence of syphilis, and did not treat the patient for syphilis. Another physician might have concluded differently; the case may be regarded as questionable.

CASE 14.—No. 22145, Mr. A. K., aged fifty-four, laborer, single. Serum tested Oct. 18, 1928, gave a ± reaction with the Bordet antigen, and 3+ with the cholesterinized antigen, while the Kahn and Hinton tests were negative. The diagnosis was arthritis. Two previous specimens from this patient gave reactions with the Kahn test also; No. 20923 (Oct. 3, 1928) gave ± with the Bordet antigen, 3+ with the cholesterinized antigen, ± with the Kahn test; No. 21345 (Oct. 8, 1928) gave no reaction with the Bordet antigen, ± with the cholesterinized antigen, + with the Kahn test. A succeeding specimen No. 22356 (Sept. 12, 1929) gave a 2+ reaction with the cholesterinized antigen only, and a negative Kahn test. Patient received treatment at a venereal disease clinic.

CASE 15.—No. 12385, Mrs. E. D., aged twenty-two, housewife. Serum tested May 14, 1929 gave 3+ reaction with Bordet antigen and 4+ with the cholesterinized antigen, while the Kahn and Hinton tests were negative. Two previous specimens were received: No. 10731 (April 24, 1929) which gave a 3+ reaction with the Bordet antigen, and 4+ with the cholesterinized antigen; and No. 11416 (May 2, 1929) which gave 2+ with the Bordet antigen and 4+ with the cholesterinized antigen. The patient was pregnant, and has been receiving treatment in a venereal disease clinic since that time. The baby's blood test No. 21203 (Aug. 28, 1929) was 2+ with the Bordet antigen and 4+ with the cholesterinized antigen. The Kahn test was negative on No. 10731, No. 11416, and No. 21203.

CASE 16.—No. 18967, I. N., child, aged eight years. Serum tested Aug. 1, 1928 gave + reaction with the Bordet antigen only, while the cholesterinized antigen, the Kahn and Hinton tests were negative. Two previous specimens were examined: No. 11736 (May 7, 1929) and No. 17886 (July 19, 1929) giving 3+ reactions with the Bordet antigen only; two succeeding specimens No. 22147 (Sept. 9, 1929) and No. 24857 (Oct. 7, 1929) gave reactions with the Bordet antigen only. These four specimens were negative with the cholesterinized antigen and the Kahn test. The child is now under treatment for syphilis. It has not been possible to obtain a specimen of blood from the parents of this child.

Of these 16 cases, therefore, 7 (Nos. 3, 6, 7, 9, 10, 11, 12) may be considered nonspecific; 2 (Nos. 8 and 13) as questionable; 4 (Nos. 5, 14, 15, and 16) as syphilitic; and from 3 (Nos. 1, 2, and 4) no further specimens or information were available. Six of the 7 nonspecific reactions were partial (+ or ±), while the seventh serum gave a 2+ reaction. This was confirmed by a second specimen which gave a ± reaction with the cholesterinized antigen.

SUMMARY

Below is a comparison of the results of the tests in this laboratory, and those of the other workers mentioned in this paper.

PERCENTAGES OF AGREEMENT BETWEEN THE WASSERMANN, KAHN AND HINTON TESTS

Cheever (Boston Dispensary)	71.7 %	2026 serums
Smith (Private practice)	65.9 %	176 "
Ferguson and Greenfield	88.5 %	460 "
Rochester Health Bureau Laboratories	87.35%	2000 "

PERCENTAGES OF AGREEMENT BETWEEN WASSERMANN AND HINTON TESTS

Hinton	89.1 %	506 serums
Munter (Peter Bent Brigham)	90.6 %	2331 "
Smith (Massachusetts General)	80.2 %	1759 "
Ferguson and Greenfield	90.0 %	612 "
Osmond	91.6 %	500 "
Jones	97.75%	400 "
Todd	96.84%	728 "
Rochester Health Bureau Laboratories	90.1 %	2000 "

PERCENTAGES OF AGREEMENT BETWEEN KAHN AND HINTON TESTS

Smith (Private practice)	74.43%	176 serums
Ferguson and Greenfield	92.4 %	460 "
Osmond	93.0 %	201 "
Rochester Health Bureau Laboratories	92.2 %	2000 "

PERCENTAGES OF AGREEMENT BETWEEN KAHN AND WASSERMANN TESTS

Ferguson and Greenfield	93.0 %	460 serums
Rochester Health Bureau Laboratories	92.4 %	2000 "

CONCLUSIONS

The Hinton glycerol-cholesterol agglutination reaction compares favorably with the Kahn precipitation test in treated cases of syphilis, in untreated cases of syphilis and in cases with suggestive histories. It is as simple to perform as the Kahn test and perhaps easier to read. The Kahn test has the advantage of requiring less time to complete the test.

The New York State Wassermann test is more sensitive in treated cases than either the Hinton or Kahn test. This greater sensitivity far more than offsets its rare, nonspecific partial reactions. The Hinton test is much easier to perform, and requires fewer and less expensive reagents and apparatus than the Wassermann test.

The Hinton test might be substituted for the Kahn test but not for a sensitive, carefully standardized Wassermann technic.

Note on Interpretation of Results of Hinton Tests.—The objective reading of the results of the Hinton Tests recorded in this paper is thought to be entirely in accord with the practice in Dr. Hinton's laboratory. In reporting and evaluating the results, however, a method of interpretation is employed by Dr. Hinton which has not been followed in this study. Dr. Hinton, who has seen the manuscript of this paper, has offered the criticism that some differences in the percentages of agreement among tests and in the correlation of results with clinical histories might have been obtained if his system of interpreting the results had been used. The following paragraphs, quoted by permission, from a letter from Dr. Hinton dealing with this question are set forth here in justice to his point of view:

> "The changes observed in the three tubes containing the individual specimen are recorded in a column as – (negative), W (weak reaction), M (moderate reaction), or S (strong reaction), as the case may be. In a separate column we indicate the significance which these readings have as to the presence or absence of syphilis, and this is our 'interpretation.'
>
> "The reactions observed and recorded as – in the three tubes are interpreted as negative. Reactions observed and recorded as W in one

or more tubes, but no stronger, are interpreted as doubtful. Reactions observed and recorded as M or S in any or all of the tubes are interpreted as positive.

"Our 'interpretation' is based on a careful clinical study of many cases and is the report which is given to the clinician as positive, negative, or doubtful. Thus, the clinician gets no idea of the readings, although we have indicated in our records the intensity of the reaction as W, M, or S. Our reason for reporting in this way is that we imply no quantitative significance by these terms, inasmuch as we have found them insignificant in therapeusis, or in diagnosis except to indicate the presence of syphilis."

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CLINICAL AND SEROLOGIC COMPARISON OF THE MICROSCOPIC SLIDE PRECIPITATION TEST FOR SYPHILIS AND THE WASSERMANN TEST WITH THE SAME ANTIGEN*

By B. S. Kline, M.D., and S. Littman, M.D., Cleveland, Ohio

IN A SERIES of 9000 microscopic slide precipitation tests and somewhat fewer Wassermann tests with the same antigen, the precipitation test was found to be more sensitive than the complement-fixation test in all stages of syphilis.

The microscopic slide precipitation test for syphilis employed in this study was described by Kline and Young.[1] It is easier to perform accurately than the Kahn tube test. It is simpler in detail and requires less serum, less apparatus and less time. The reactions are somewhat stronger in the slide test and the results, magnified about 100 times by the microscope, are much easier to read than those of the Kahn test.

The Wassermann test was done by the Cleveland method.[2] It employs overnight primary incubation at 8° C. to 12° C., 5 to 10 units of amboceptor and 2 units of complement. In a comparative study of 1000 tests by Lyne[3] the Cleveland-Wassermann test and the Kolmer-Wassermann test were found to be of equal sensitivity and specificity.

The antigen employed for both precipitation and complement-fixation tests was that described by Kline.[4] It is a lipid obtained from chilled absolute alcoholic extract of beef heart powder by precipitation in acetone at 50° C. to 37° C.

The emulsions for the precipitation test prepared from Kline antigen, in contrast to Kahn antigen dilutions, are (a) relatively stable, retaining their antigenic properties undiminished for two days; (b) more sensitive than the standard Kahn antigen dilutions;[4, 5] (c) always free of clumps with non-syphilitic sera at low temperatures as at ordinary room temperature; (d) uniform in quality and quantity containing numerous discrete fine particles resembling those of a negative reaction, whereas Kahn antigen dilutions vary in content and contain clumps of undispersed particles resembling those of a positive reaction.

The emulsions for the Wassermann test were prepared by proper dilution of the emulsions for the precipitation test.

Table I gives the clinical evaluation of the two tests:

*From the Laboratory Department and the Department of Syphilology of the Outpatient Clinic, Mount Sinai Hospital.
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TABLE I

CLINICAL EVALUATION OF MICROSCOPIC SLIDE PRECIPITATION TESTS AND WASSERMANN TESTS WITH THE SAME ANTIGEN

	TOTAL TESTS	TESTS IN CASES WITH HISTORY UNOBTAINABLE, DATA INSUFFICIENT, OR SYPHILIS DOUBTFUL	AGREEMENT	TOTAL DISAGREEMENT	DISAGREEMENTS												
					FALSE NEGATIVES							POSITIVE REACTIONS—NO EVIDENCE OF SYPHILIS					
					PRIMARY		TREATED SYPHILIS						SLIDE TEST		WASSERMANN TEST		CLINICAL DIAGNOSIS
					UNTREATED	TREATED	SECONDARY	TERTIARY	C. N. S.	CONGENITAL	NOT DETERMINED	TOTAL	SENSITIVE	VERY SENSITIVE	SENSITIVE	VERY SENSITIVE	
VERY SENSITIVE SLIDE TEST	9001 (1861 syphilitic sera ± to ++++ in slide, Wassermann or both tests 20.85%)	76	8829 98.92%	96 1.08%	0	3 0.033%	21 0.24%	55 0.61%	3 0.033%	1 0.01%	3 0.033%	10 0.11%	++ + − + 0 + ± + + −	++++ ++ ++ ++ ++ ++ ++ ++ ++ ++	− − − − 0 − − 0 − −	− ± 0 ± − − − 0 − −	Cervicitis, vaginal discharge Chronic pelvic inflammatory disease Pregnancy Acute pharyngitis Lysol poisoning, mitral stenosis Lysol poisoning Bartholin cyst Cirrhosis of liver Neurasthenia Diabetes mellitus
VERY SENSITIVE WASSERMANN TEST (A)	8758	76	8421 96.99%	261 3.01%	3 0.03%	4 0.05 %	70 0.81%	151 1.74%	7 0.08 %	11 0.13%	9 0.10 %	6 0.07%	− − − − 0 −	− − − ± − −	− +,++ − − 0 −	+++ ++ +++ ++ ++ +++	Miliary tuberculosis Pregnancy Neurasthenia Influenza Pleurisy with effusion (TBC ?) Carcinoma of bladder
SENSITIVE SLIDE TEST	8897	76	8649 98.05%	172 1.95%	0	4 0.04 %	42 0.48%	109 1.24%	4 0.04 %	6 0.07%	6 0.07 %	1 0.01%	++	++++	−	−	Cervicitis, vaginal discharge
SENSITIVE WASSERMANN TEST (B)	8513	76	7769 92.08%	668 7.92%	3 0.04%	6 0.07 %	160 1.90%	426 5.05%	16 0.19 %	26 0.31%	29 0.34 %	2 0.02%	− −	− −	+++ ++	+++ ±	Miliary tuberculosis Pleurisy with effusion (TBC ?)

(a) About 35% of tests with 0.2% cholesterinized antigen; about 65% with 0.3% cholesterinized antigen.
(b) About 35% of tests with noncholesterinized antigen; about 65% with 0.06% cholesterinized antigen.
± to ++++ any test in syphilitic serum = agreement.
Negative test in syphilitic serum, other test ± to ++++ = false negative.
++ to ++++ any test in nonsyphilitic serum = false positive.

Table I shows the greater sensitivity of the microscopic slide precipitation test in all stages of syphilis. Positive reactions in cases showing no evidence of syphilis were very few and probably due to technical errors.

Table II shows the comparative sensitivity of the two tests in primary syphilis before treatment.

TABLE II

COMPARISON OF SLIDE PRECIPITATION TEST AND WASSERMANN TEST IN CASES OF PRIMARY SYPHILIS BEFORE TREATMENT

DATE	SLIDE PRECIPITATION TEST		WASSERMANN TEST		CLINICAL DATA
	SENSITIVE	VERY SENSITIVE	SENSITIVE	VERY SENSITIVE	
11/11/27	+	++	−	−	Case 1.—(J. A.) Chancre seven days' duration
2/16/29	−	±	−	−	Case 2.—(J. C.) Chancre seven days' duration. Fontana stain negative for Spirocheta pallida, positive for Spirochete vincenti
2/21/29	+	++	−	−	
4/ 1/29	++++	++++	++++	++++	Typical chancre, enlarged inguinal nodes on right side, maculopapular syphilides
4/ 6/29	++++	++++	++++	++++	

Table II shows the greater sensitivity of the precipitation test in primary syphilis before treatment.

TABLE III

COMPARISON OF SLIDE PRECIPITATION TEST AND WASSERMANN TEST IN A CASE OF TREATED SECONDARY SYPHILIS

DATE	SLIDE PRECIPITATION TEST		WASSERMANN TEST		CLINICAL DATA
	SENSITIVE	VERY SENSITIVE	SENSITIVE	VERY SENSITIVE	
5/21/27					Case W. D. Chancre two months' duration. Wassermann test reported positive in May at City Hospital. Patient received three injections at City Hospital
6/ 2/27		++++	++++		
7/11/27		++++	+++		
9/23/27	+	++++	−	+++	
10/20/27	+	+++, ++++	−	−	
11/17/27	+	++	−	−	
2/18/28	+	+	−	−	
8/23/28	++++	++++	++	++++	Clinical recrudescence
9/13/28	++++	++++	++	++++	
10/25/28	+++	++++	−	++	
11/20/28	+	++	−	−	
4/11/29	±	++	−	−	
4/18/29	±	++	−	−	
5/ 2/29	−	±, +	−	−	
8/10/29	±	+	−	−	

Table III is a representative protocol showing the comparative sensitivity of the two tests in secondary syphilis.

Table III shows the greater sensitivity of the slide precipitation test in secondary syphilis.

Table IV illustrates the comparative sensitivity of the two tests before and following treatment early in syphilis.

TABLE IV

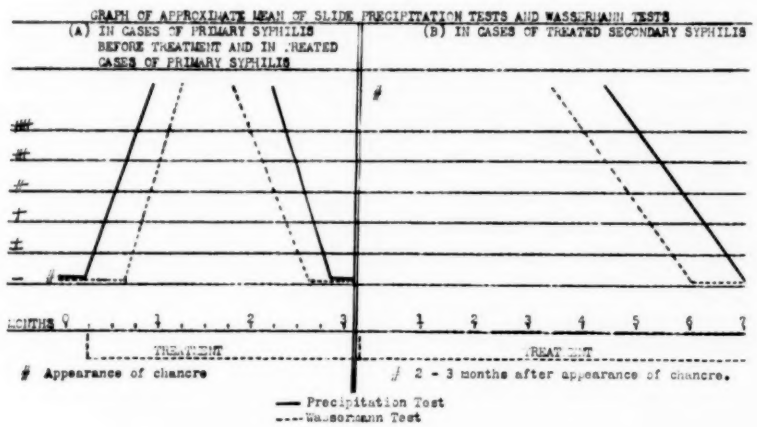
GRAPH OF APPROXIMATE MEAN OF SLIDE PRECIPITATION TESTS AND WASSERMANN TESTS
(A) IN CASES OF PRIMARY SYPHILIS BEFORE TREATMENT AND IN UNTREATED CASES OF PRIMARY SYPHILIS
(B) IN CASES OF TREATED SECONDARY SYPHILIS
MONTHS
TREATMENT
UNTREATED
Appearance of chancre
// 2 - 3 months after appearance of chancre.
Precipitation Test
Wassermann Test

Table IV shows the greater sensitivity of the precipitation test in primary syphilis before treatment and following it, and in secondary syphilis following treatment.

Table V is a representative protocol showing a comparison of the two tests in treated syphilis.

TABLE V

PROTOCOL SHOWING GREATER SENSITIVITY OF SLIDE PRECIPITATION TEST IN CASES OF TREATED SYPHILIS (5/11/28)

PATIENT	STAGE OF SYPHILIS	SLIDE PRECIPITATION TEST		WASSERMANN TEST	
		SENSITIVE ANTIGEN	VERY SENSITIVE ANTIGEN	SENSITIVE ANTIGEN	VERY SENSITIVE ANTIGEN
D. B.	3	++++	++++	+++	++++
G. W.	3	++	+++, ++++	–	++
B. B.	2	–	–	–	–
M. L.	Cong.	+++, ++++	++++	++	++++
A. M.	3	++++	++++	–	++++
T. S.	3	++	+++	–	+++
C. B.	3	++++	++++	+++	++++
A. G.	3	+++	+++, ++++	–	–
G. P.	3	++++	++++	++	++++
M. C.	3	+++, ++++	++++	++	++++
J. N.	3	++	++++	–	++
L. C.	3	++	+++	++++	++++
L. A.	2	+	++, +++	–	+++
Total positive reactions		11	12	6	11
Total doubtful reactions		1	0	0	0
Total negative reactions		1	1	7	2
Total tests		13	13	13	13

The following table shows the serologic comparison of the two tests:

TABLE VI

COMPARISON OF 8708 MICROSCOPIC SLIDE PRECIPITATION TESTS AND WASSERMANN TESTS WITH THE SAME ANTIGEN

	SLIDE TESTS AND WASSERMANN TESTS WITH VERY SENSITIVE ANTIGEN EMULSION					SLIDE TESTS AND WASSERMANN TESTS WITH SENSITIVE ANTIGEN EMULSION				
			DISAGREEMENT					DISAGREEMENT		
	AGREEMENT	RELATIVE AGREEMENT	POSITIVE SLIDE, NEGATIVE WASSERMANN	POSITIVE WASSERMANN, NEGATIVE SLIDE	TOTAL TESTS	AGREEMENT	RELATIVE AGREEMENT	POSITIVE SLIDE, NEGATIVE WASSERMANN	POSITIVE WASSERMANN, NEGATIVE SLIDE	TOTAL TESTS
Tests	7968	509	166	65	8708	7530	633	317	43	8523
Per cent	91.50	5.84	1.91	0.75		88.35	7.43	3.72	0.50	
Tests	8477		231		8708	8163		360		8523
Per cent	97.34		2.66			95.78		4.22		

Evaluation of results:—
Positive Reaction = ++++, +++, and ++.
Doubtful Reaction = + and ±.
Agreement = positive, negative or doubtful by both methods.
Relative agreement = positive or negative by one method and doubtful by the other.
Disagreement = positive by one method and negative by the other, and vice versa.

Table VI shows the greater number of positive precipitation tests and the closer agreement of the very sensitive tests with each other than the sensitive tests with each other.

DISCUSSION

The Wassermann test requires the careful preparation of five main ingredients: antigen, patient's serum, complement (from guinea pig), red blood cell suspension (from sheep), and amboceptor (from rabbit immunized against sheep red blood cells). The precipitation test requires but two main ingredients, antigen and patient's serum.

The precipitation test is much simpler in detail than the Wassermann test and offers less opportunities for error and less technical difficulties. The precipitation test in other words is easier to perform accurately than the Wassermann test. Furthermore, the precipitation test is more sensitive than the Wassermann test with the same antigen. This is apparently due to the fact that concentrated ingredients are used whereas in the Wassermann test the antigen is greatly diluted and the serum somewhat diluted before the reaction occurs.

The sensitive slide test, more sensitive than the very sensitive Wassermann test, with but one false positive reaction in 8897 tests, is especially valuable in that a ++ or stronger reaction is practically diagnostic of syphilis. The very sensitive slide test is especially valuable in that when negative it rules out syphilis except in a very small percentage of cases.

CONCLUSION

In a series of 9000 microscopic slide precipitation tests and somewhat fewer Wassermann tests with the same antigen, the precipitation test was found to be more sensitive than the complement-fixation test in all stages of syphilis.

We wish to acknowledge the excellent technical assistance of Miss M. G. Bowman, A.B., in the performance of the majority of the tests reported in this study.

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A ROUTINE BLOOD CHEMISTRY UNIT*

By E. G. Schmidt, Ph.D., Baltimore, Md.

THE advent of clinical chemistry as an important diagnostic factor in medicine and surgery necessitates simplified methods and apparatus for the routine determination of the various constituents of the blood and urine. In the hospital where a number of individuals work interchangeably, particularly in regard to emergency examinations, a permanent unit for the routine blood chemistry determinations which is practically error-proof is very desirable. During the last few years a unit has been in use in the Mercy Hospital laboratory which has aided materially in promoting efficiency, economy, and accuracy of results, with the consequent elimination of errors and of confused blood specimens. Essential details of the set-up are illustrated in Fig. 1 which is practically self-explanatory and which was kindly taken for us by Mr. Carl Clark of the Art Department of the University of Maryland School of Medicine.

The essential feature of the apparatus, besides the burette system, is several rows of solid glass rods driven firmly into holes which have been bored into the solid board background at a slight angle. Upon these solid glass pegs, which are about 5 inches long and $\frac{3}{8}$ inch in diameter, are placed the

*From the Biochemical Laboratory of Mercy Hospital and the Department of Biological Chemistry of the University of Maryland School of Medicine, Baltimore, Maryland.
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various tubes used in the blood chemistry determinations. Each row of tubes, reading from the top downward, is labeled at the left of the board, e.g., non-protein nitrogen, creatinine, uric acid, "urea incubation," "urea nesslerization," blood sugar, etc. Below the lip of each tube is placed a strip of adhesive tape upon which is written with a glass-marking pencil the number of the tube. Each row of these tubes is numbered from left to right in numerical sequence; the last tube on the right is marked "S" and is used for the standard solution. In the solid filter-board a number of holes are bored, and numbered to correspond with its blood specimen and the glass peg above it.

The complete blood chemistry is carried out in the following manner: A number of small 2 oz. wide-mouth bottles containing 1 c.c. of a 1 per cent solution of lithium-, potassium-, or sodium oxalate are baked in an oven overnight and as a result the oxalate is left in a finely divided condition which

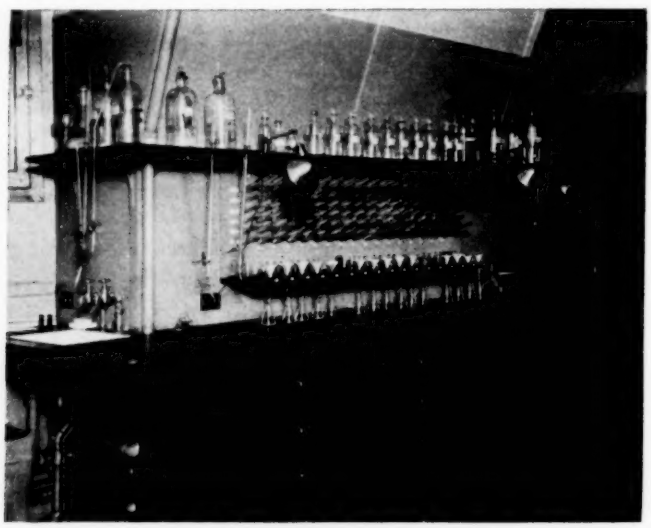

Fig. 1.

materially enhances its anticoagulative effect. About 6 c.c. of blood is obtained from each patient in the oxalate bottles in the usual manner. The specimen bottles labeled with the name and number of the patient are arranged in a row before the apparatus, one bottle on each filter hole. Usually 5 c.c. (1 volume) of blood are accurately pipetted into an Erlenmeyer flask to which 40 c.c. (8 volumes) of 0.12 N. sulphuric acid[1] are immediately added from the burette at the left of the board. The contents of the flask are shaken with a rotary motion and some of the solution is drawn into the pipette and then allowed to drain out again. Thus each blood is acidified and its empty blood container placed in sequence at the back of the board. The pipettes are then blown out and placed on glass wool in a large jar containing a solution of chromic acid cleaning solution.[2] Each Erlenmeyer flask is now given a final inspection and if all the blood is hemolyzed then 5 c.c. (1 volume) of 10 per cent sodium tungstate is added from the burette just to the

left of the board. The flasks are given a vigorous rotary whirl to aid protein precipitation; but it is not necessary to stopper the flasks when shaking as in the original Folin-Wu method.[3] The contents of the flask are allowed to stand about five minutes, with an occasional shake, and then poured upon the filters which are also in numerical sequence. If the bloods were completely hemolyzed before the addition of the sodium tungstate the filtrates will invariably come through water-clear.

The following analytical procedures are used: blood sugar by the method of Benedict,[4] nonprotein nitrogen and creatinine according to Folin-Wu,[3] amino-acid nitrogen according to Folin[3] and to Schmidt,[5] uric acid by the method of Benedict,[6] and the blood urea by the method of Karr.[7] This direct nesslerization method for the determination of blood urea will be described somewhat in detail, because it is an essential part of the blood chemistry and fits in well with the above described unit.

Into each tube numbered in red and labeled "urea incubation" are placed 5 c.c. of the corresponding protein-free filtrates and into the standard urea tube marked "S" is placed 5 c.c. of the standard urea solution containing 0.075 mg. urea nitrogen. Two drops of a phosphate buffer solution[8] are added to each tube. Then 1 c.c. of a concentrated glycerol urease preparation is diluted to 10 c.c. with distilled water and 1 c.c. of the enzyme solution is added to each tube.[8] The diluted urease solution should be prepared daily, but the concentrated glycerol solution retains its full activity for at least a year. The tubes are now incubated for five minutes at 40° to 50° C., and the contents are then quantitatively transferred to the corresponding tubes numbered in blue and labeled "urea nesslerization." This quantitative transfer is carried out in such a manner that the contents of the tubes now have a volume of about 20 c.c. Now 2.5 c.c. of Nessler's reagent[9] are added to the standard urea tube and the contents diluted with distilled water to 25 c.c. and mixed by inversion. The solution is now placed in the cups and the colorimeter adjusted for proper readings. Then 2.5 c.c. of Nessler's reagent are added to tube Number 1 and the contents are diluted to 25 c.c., mixed by inversion, and matched at once in the colorimeter. The filtrates are nesslerized and matched singly because a precipitate rapidly forms upon nesslerization which tends to vitiate the results. The tubes are immediately rinsed out with distilled water and replaced upon the glass pegs in numerical order by the analyst who carried out the determination. The "urea incubation" and "urea nesslerization" tubes must never be used interchangeably, and if Nessler's reagent or any mercury solution gets into the incubation tubes, sufficient mercury will be adsorbed to inactivate[10] the urease and as a result the urea will be low and incorrect. When this happens the tubes must be rinsed out with dilute acid and with water several times in order to completely remove the adsorbed mercury.

SUMMARY

A mechanical unit is described for the routine determination of blood chemistries in the hospital laboratory. The use of this apparatus in the deter-

mination of blood urea by the direct nesslerization method is described somewhat in detail.

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A NEW MICROCOLORIMETER*

By A. G. Sheftel, M.D., New York City

THE number of physicians who are doing their own laboratory work, especially blood sugars, is increasing rapidly. A colorimeter is required for most of the blood analyses, but the standard colorimeters are quite expensive, and as most physicians need a colorimeter for only one or two items in which they are particularly interested, they use either the dilution method (principle of Sahli hemoglobinometer) or a series of colored tubes filled with colored liquid and used as standards. The disadvantage of the dilution method is that the dilution must be done very slowly, drop by drop, and there is always a certain loss of liquid entailed by the necessary shaking up of each dilution. If a few drops of water are involuntarily added, making the liquid to be examined lighter than the standard, the entire procedure must be repeated. As to the method which employs standards made of a series of tubes filled with colored liquid, the disadvantage is that it is almost impossible to have a liquid standard which does not fade somewhat with time. The most ideal standards are therefore those which are freshly made up and each time from the substance to be examined, or one made out of colored glass which will retain its color permanently.

The colorimeter herein described, like all standard colorimeters, is based on Beers' law which states that light in passing through a colored medium is absorbed in direct proportion to the concentration of colored substance, and it consists of two opaque tubes (Nos. 1 and 2) of the same diameter, both having transparent glass bottoms. Underneath these tubes there is a mirror (4) reflecting light. Tube 1 is connected at the lower end with a 1 c.c. tuberculin hypodermic syringe,[3] by means of which the liquid to be examined can be withdrawn and then added at will, thus lowering or raising the level of the liquid in Tube 1, and so decreasing or increasing the intensity of color of

*From the Metabolism clinic of Mt. Sinai Hospital, New York.

the liquid when examined from the top of the tube. When the color in Tube 1 matches the color of the standard, the percentage of the unknown substance can be ascertained from the divisions on the syringe. Tube 2 is for a standard solution, which can be made fresh each time from the substance to be examined, or the tube can also be made with a colored glass bottom to be used as a permanent standard. Directly under both tubes a yellow colored glass (5) can be inserted to facilitate the matching of colors when the new Folin-micro method for sugar in blood is used. This yellow glass acts as a filter in removing the yellow color, which is due to an excess of potassium ferricyanide in the reagent.

The colorimeter is used in the following manner. For instance we want to test sugar in the blood. After the substance to be tested is transformed into a colored compound and properly diluted, 2 c.c. of this liquid is poured into Tube 1 and 2 c.c. of the freshly made standard, representing 0.1 per cent

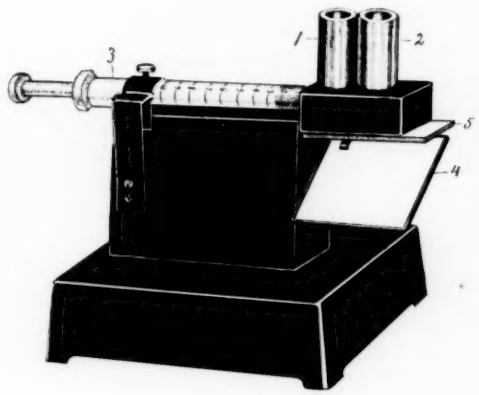

Fig. 1.

sugar, is poured into Tube 2, or a permanent glass standard may be used instead. If the solution to be tested is lighter than the standard, this indicates that the blood contains less than 100 mg. of sugar per 100 c.c. In this case add another c.c. of the unknown colored compound to Tube 1. By moving out the piston of the hypodermic syringe, the level of the liquid is lowered and the depth of the color diminished until both colors match. All shades can be obtained representing from 0.067 per cent to 0.1 per cent sugar. In case the solution is still lighter than the standard, after having added 1 c.c., this indicates that the blood contains less than 0.067 per cent sugar and so we add still another c.c. of the unknown solution to Tube 1, and by moving the piston again, we can obtain all shades representing from 0.05 per cent to 0.067 per cent sugar in the blood. In case there is more than 0.1 per cent sugar in the blood, the solution to be tested will be darker than the normal standard. In this case by moving the piston out we can obtain all necessary shades representing from 0.1 per cent to 0.2 per cent sugar. After pulling out the piston until we have removed 1 c.c. of fluid, if the liquid is still darker than the standard, it shows that there is more than 0.2 per cent sugar present,

and so we can use either a 0.2 per cent standard, or if we only want to use the 0.1 per cent standard, we push back the liquid into Tube 1 with the piston, and by means of a pipette withdraw 1 c.c. of liquid then add 1 c.c. of water, mix well with the pipette, and move piston in and out several times to insure proper dilution. Then by moving the piston out slowly we obtain all shades representing from 0.2 per cent to 0.4 per cent sugar, etc.

This colorimeter gives sufficiently accurate results for clinical work and can be made up very inexpensively to enable every physician to do any work requiring a colorimeter, regardless of whether he needs a colorimeter for one item or several.

310 West Seventy-Second Street.

A GRADUATED TEST TUBE COLORIMETER*

By Arthur T. Brice, Jr., B.A., Florence, S. C.

THE instrument to be described was developed through the necessity of having available in the laboratory a suitable substitute for the standard instrument in the event that the latter should become accidentally damaged. Such an occurrence at a remote distance from optical repair facilities might easily have caused a disastrous tie-up of the clinical laboratory routine. A secondary motive was the possibility of offering to the young intern preparing to make a start in general practice an inexpensive item of equipment which might enable him to carry his knowledge of blood chemistry with him into his work, it having been our observation that the majority of young doctors refrain from installing the necessary equipment for this purpose solely on account of the expense of the colorimeter, which they estimate that they will have need for only occasionally. The necessary glassware and reagents for the most commonly employed blood chemistry determinations cost but a very few dollars, while the colorimeter which may be employed but once or twice a month will run close to a hundred and may not pay for itself in a very long time.

The instrument which we have evolved, and which we have called a Graduated Test Tube Colorimeter, was developed without any knowledge of the Denison Laboratories colorimeter, described by Peebles and Lewis,[1] and without any design to improve on this instrument, which, however, may be regarded as its direct lineal predecessor. It differs from the Denison instrument in several important features. The tubes of the Denison instrument being placed in a parallel position, it is impossible to view the colored solutions in both simultaneously, a factor of considerable importance for accurate and easy color matching. The ground glass diffusion screen employed in the Denison instrument also admits to the body of the instrument a wave front of

*From the Clinical Laboratory of the McLeod Infirmary, Florence, S. C.
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light of several times the square area of the cross-section of the tubes themselves, making extreme precision of construction necessary in order to satisfactorily eliminate disturbing reflected rays of light. In our instrument the tubes are aligned at an angle with each other making it easily possible to view the solutions in both simultaneously, and, the same bit being used for boring the tube barrels, two exactly equal pencils of light only are admitted to the body of the instrument. The work done with the Denison colorimeter, however, stands as a record of the reliability of the general principles underlying our instrument.

Myers[2] has described an inexpensive test tube colorimeter employing the principle of dilution, but this instrument, as he has himself subsequently pointed out,[3] suffers from several practical disadvantages, the principal one of which is that fairly large volumes of liquid are required in order to secure accuracy. The author believes that his instrument offers a more perfect optical system than Myers' side to side comparison, in that the whole volumes of

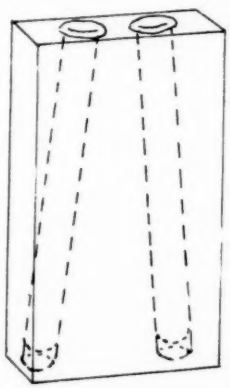

Fig. 1.

both liquids under comparison are under observation simultaneously, and no other volumes of the same or similarly colored liquids are in the field of vision.

The device consists of a block of wood through which two holes are bored to receive two graduated test tubes for the known standard and the unknown colored solutions which it is desired to compare. The block is preferably made from an inexpensive hardwood such as maple, though this is not necessary as soft woods are practically equally suitable. A convenient size is 32 × 76 × 165 mm. Test tubes for such a block must be carefully matched so that columns of liquids of equal height will show identical readings by the graduation marks. This matching will usually be done with sufficient accuracy by the reliable supply houses if it is specifically stated in the order for what purpose the tubes are to be employed. We have found tubes of 15 ml. capacity with minimum graduation interval of 1/5 ml. and an outside diameter of 13 mm., with the block described, to make a very compact and handy instrument.

In operation the instrument is held in one hand at a convenient reading distance from the eyes and sighted, preferably, but not necessarily, with both

eyes open, downward toward an evenly and brightly illuminated white surface such as a sheet of paper or a porcelain or enamel plate placed upon the table. A fixed setting may be employed, the liquid being poured into the tube to the desired mark and the tube then being placed in position in the block, or the liquid may be poured into the tube after it is in position in the block to any convenient height. The standard having been set in this manner, the instrument with both tubes in position is next sighted as described and the unknown solution poured into the other tube, a little at a time, sighting carefully after each addition, until an exact color match with the standard is obtained. Both tubes are then removed from the block and the readings made. One of the tubes may next be emptied and the matching process repeated any desired number of times to give a series of readings which are finally averaged and the calculation made according to the usual formulas for the standard prism types of instrument.

Fig 2.—Illustrating use of the instrument.

The essential feature of design required to make the matching of colors easy by this method consists in properly aligning the bores of the test tube holes through the block, so that both tubes may be sighted through with one or both eyes open, and with a minimum of required movement of either the head or the block. If the alignment is correct so that an absolute minimum of motion is required, the faculties may be concentrated on the color comparison, and very accurate matching may be done. By practical experiment rather than from theoretical considerations the following dimensions have been determined. The distance between the tube bores from center to center at the top of the block should be 33 mm., and the central axes of the bores should form an angle with each other of 5 degrees. The axes should of course lie in the same plane. Using an instrument of these specifications, held at average reading distance of about 13 inches from the eyes, it has been found possible to match colors satisfactorily by operators of normal or corrected vision having interpupillary distances ranging from 62 to 66 mm. As interpupillary distances beyond these limits are rarely met with, it is felt that these specifications will be most suitable for the average.

The block is best bored accurately in a lathe setting it in a frame so that the bit will bore each hole at an angle of 2.5 degrees with the central longitudinal axis of the block. In order to permit the tubes to be readily inserted and removed the holes should be of inside diameter approximately 3 mm. larger than the outside diameter of the tubes. The internal surfaces of the bores should be colored a dead black to minimize reflections and permit of only equal amounts of light to pass through the two tubes. This is easily done by dipping the whole block in a pan of dead black paint, or by pouring the paint through the bores carefully so that the entire internal surfaces are covered, and then hanging the block to drain evenly and dry. The instrument can be manufactured at a total cost of less than two dollars.

In order to carry out a fair test of the accuracy of this instrument a series of 99 comparisons with the standard prism type instrument has been run. In performing these comparisons the determinations were first made accurately with the standard instrument, making two or three readings as is customary before the final calculation. A comparison by means of the graduated test tube colorimeter was then made, of the same two colored solutions, only making but one reading from which to calculate the value as determined by it. The series includes the following determinations: 2 spinal fluid sugar by Folin-Wu technic; 3 blood iron by Wong technic; 3 blood urea nitrogen by Folin distillation technic; 4 blood performed creatinine by Folin technic; 20 blood nonprotein nitrogen by Folin micro-Kjeldahl technic; 33 blood sugar by Folin micro technic; 34 blood sugar by Folin-Wu technic. Assuming the determinations as made with the standard prism instrument to have been the true values the results of this series indicate the following factors of error: In 85 per cent of the determinations with the test tube instrument the error was less than 10 per cent. In 43 per cent of the determinations the error was less than 5 per cent. In 26 per cent of the determinations the error was less than 2 per cent. In 13 per cent of the determinations the error was less than 1 per cent. The maximum individual errors of 17 per cent and 18 per cent were found in the series in those procedures with which the technician was least familiar, spinal fluid sugar, and blood urea nitrogen. These findings indicate that in the hands of the inexperienced a maximum error of as high as 18 per cent may be obtained, but that with practice, such as matching the standard against itself a number of times before each determination, and with care, such as averaging the readings of a number of comparisons before making the final calculation, sufficient skill may easily be acquired to make the use of this instrument entirely satisfactory to the general practitioner. The plan followed of making but one reading to arrive at the factors of error reported for the test tube instrument operated to its disadvantage, so that it is safe to say that these factors are high and that a considerably greater degree of accuracy would be found should the instrument be used only after practice and with care as prescribed. The mean average error of the graduated test tube colorimeter in the series reported was minus 1.542 per cent. The instrument is therefore offered.

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A NEW TYPE OF MASK FOR THE STUDY OF RESPIRATORY EXCHANGE OF THE DOG*

BY ASHLEY W. OUGHTERSON, M.D.,† NEW HAVEN, CONN.

IN THE study of the respiratory exchange of the dog, one of the chief obstacles has been the development of an efficient mask. A satisfactory type of mask would be one in which the dead space was reduced to a minimum, that could be quickly and easily adjusted without discomfort to the dog and at the same time would not leak. In the study of the basal metabolic rate the factor of making the dog comfortable is almost as important as the elimination of leaks.

Fig. 1.—Mask adjusted to dog.

Several types of masks have been devised by previous investigators. Among the earliest of these were those made of plaster of Paris for each individual animal according to the contour of the face. The mask was then covered with paraffin and an air-tight connection made by the use of plastacene or a similar material. This was difficult to hold in place and uncomfortable. Boothby and Sandiford described a mask which enclosed the entire head of the animal, the air-tight connection being made by means of wrapping the mask and neck of the animal with a strip of rubber dental dam 5 inches wide and 10 feet long. With this procedure it was necessary to shave the neck of

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the dog and it was difficult to adjust the wrapping around the neck with comfort. This type of mask was also used by Kitchen. The mask used by Kunde was simple and efficient although there was considerable dead space. It consisted of a metal cylinder across one end of which was stretched a sheet of rubber dental dam with a small hole in the center. A circumscribed area be-

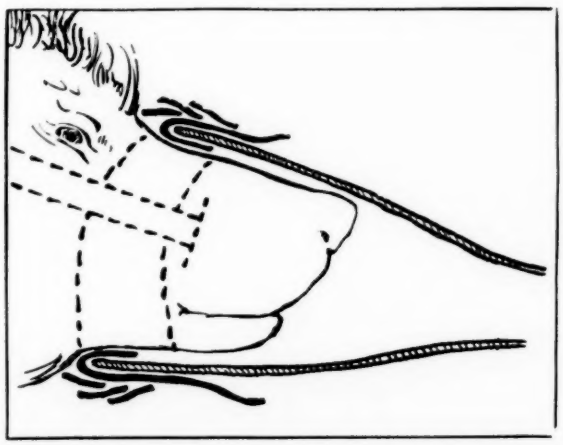

Fig. 2.—Shows principle of folded rubber contact.

Fig. 3.—Sheet and roll of rubber dental dam with mask.

tween the corners of the mouth and the eyes was shaved and the muzzle of the animal inserted into the hole in the rubber dental dam. When widely varying types (e.g., short-nose bulldogs) were studied, some difficulty was experienced in holding the mask in place. Blalock devised a rubber mask the rim of which could be inflated. The muzzle of the dog was first wrapped with a

strip of dental dam and the mask was then stretched over this and inflated. The mask was devised for use with the animal lying on its back and considerable difficulty was experienced in holding the mask in place with the animal on its side, which position is necessary to obtain the basal metabolic rate. It was also not as comfortable to the dog as the one here described.

Fig. 1 shows the mask fitted to the dog and Fig. 2 shows the principle of the overlapping rubber to rubber contact. Fig. 3 shows the sheet of rubber dental dam with a hole cut in the center. The size of the hole should be varied according to the dog so as to give a snug fit yet not be tight enough to cause edema of the tissue. It is not necessary to shave the muzzle, but a circumscribed area between the corners of the mouth and the eyes should be clipped with fine clippers. The sheet of rubber dam is drawn on over the muzzle and adjusted behind the corners of the mouth. The mask is then slipped on and secured with the straps behind the head. The edges of the rubber dam are then turned down and wrapped underneath the straps with a roll of rubber dam 1½ inches wide and 3 feet long. The connection with the spirometer is made with an ordinary ground brass union. The straps are of assistance in holding the mask in place and are particularly useful during the training period. This mask has been used successfully in over five hundred determinations of the basal metabolic rate. From the illustrations, it will be seen that the dead space is reduced to a minimum. The mask is manufactured by the Baumann Rubber Company of New Haven, Connecticut.

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SIMPLE METHODS OF PRESERVING CULTURE MEDIA

By Herman A. Heise, M.D., Uniontown, Pa.

THE method of preserving culture media described here has been successfully employed in the laboratory of the Uniontown Hospital for the last ten years, and is recommended because of its simplicity and the fact that it requires neither ice box nor difficult technic.

The culture media is tubed and stoppered in the ordinary manner, and the tubes then put into quart mason jars, fifteen to twenty of the ordinary

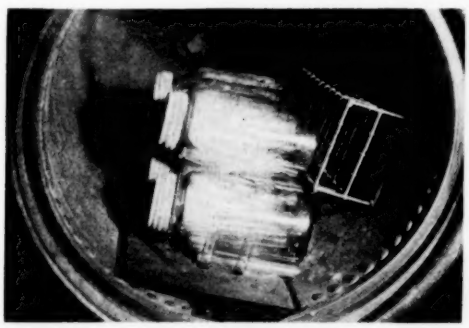

Fig. 1.—Method of arranging jars of Loeffler's blood serum in autoclave ready for sterilization.

Fig. 2.—Completed culture media.

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sized tubes 120 by 16 mm. being placed in each jar. Needless to say, jars of other sizes can be obtained to accommodate tubes of different measurements. If solid culture media is to be made the tubes are pressed against one side of the jar so that all lie parallel. The lid is screwed on loosely and the jars arranged in the autoclave, tilted at the proper angle for slants. The sterilization is performed in the usual manner except that the time is increased about 50 per cent, and the jars and autoclave are allowed to cool without having the contents disturbed. Not until the slants have cooled are the lids screwed on firmly and the jars may then be placed on shelves. This method is particularly applicable to the making of Loeffler's blood serum as smooth, moist slants free from bubbles are obtained, possibly because heating and cooling are delayed by the jars. It is very important, however, not to firmly fasten the lids tightly until the media has cooled, or bubbles will form.

In preparing liquid media for blood cultures the use of citrate bottles has been found most convenient. The media is placed in the bottles, the cover put on loosely, the spring catch not being pressed down in the locked position. A piece of cloth about six inches square is fastened over the head of the bottle with a rubber band and the media then sterilized in the autoclave. After sterilization has been completed the spring catch is pulled down sealing the bottle. Before use, the cloth covering of the bottle is soaked with an antiseptic to further guard against contamination.

ADVANTAGES OF THE METHOD

Culture media prepared in this manner remain sterile indefinitely, an ice box is not necessary, the media does not dry out, slants are uniform, and the technic is simple. No outlay of money for jars has been necessary at this institution, since these jars are continually brought to the laboratory as specimen containers.

AN IMPROVED PIPETTE MANIPULATOR*

By D. C. B. Duff, M.A., Vancouver, B. C.

THE ordinary laboratory method of measuring out small quantities of bacterial antigens or other fluids in pipettes which are manipulated by mouth and hand are unsatisfactory for two reasons.

1. The possibility of infection of the operator where live antigens are used is great, and may be pointed out as a possible source of the many laboratory infections by organisms such as Br. abortus.

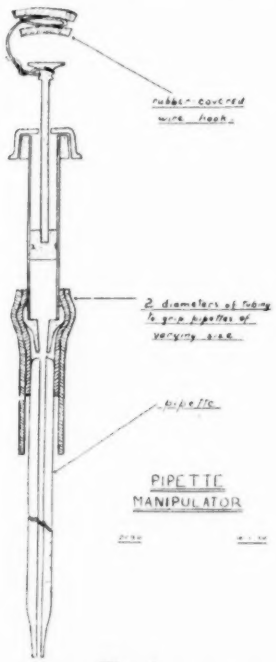

Fig. 1.

2. Even in the hands of experienced operators, accuracy in delivery by gravity is frequently sacrificed in the interests of speed.

Carpenter, Boak, and Chapman[1] have described an instrument extensively used for this purpose on the continent, and known as a rheometer. This consists, essentially, of a graduated Luer syringe, to the plunger of which is attached an extension rod or piston. The plunger is normally kept in the "full" position by means of a coiled spring. The syringe is filled by pressing down the plunger and then allowing the fluid to be drawn up through a long needle

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[1]Carpenter, C. M., Boak, R., and Chapman, O. D.: J. Immunol. 17: 73, 74, 1929.

by the force of the spring. Delivery is made according to the graduations on the syringe by again pressing against the force of the spring.

Owing, however, to the fact that syringes are not as accurately graduated as are standard pipettes, and also to the fact that in the syringe the diameter of the meniscus is large, permitting errors in delivery, the rheometer offers certain disadvantages for work in which accuracy is at a premium.

A very simple alternative arrangement, which permits the use of standard pipettes, at the same time avoiding infections and allowing absolute accuracy, is here described. A Record type syringe, having a metal plunger of fairly stiff movement, is selected. Reference to the diagram explains the construction. A short length of heavy walled rubber tubing is slipped over the tip of the syringe, leaving a sufficient amount projecting to grasp and hold firmly the top of the pipette. The instrument may be adapted for use with pipettes of two different diameters by cementing in a shorter length of tubing of smaller diameter as shown, leaving at the same time a sufficient free length of the larger tubing to grasp the larger size pipette. A stiff wire is attached to the plunger and bent as shown. It is covered by a piece of tubing where the finger will come in contact with it.

In operation, a pipette is inserted into the rubber receiver. The barrel of the syringe is grasped with thumb and third finger, and the index finger is slipped between the plunger and wire guard. The finger may then be moved up or down, giving a positive manipulation both ways.

AN APPARATUS FOR THE SLOW INTRA-ARTERIAL INJECTION OF M/6 SODIUM CARBONATE SOLUTION AS AN ANTICOAGULANT IN BLOOD PRESSURE EXPERIMENTS BY MEANS OF COMPRESSED AIR*

By Walter E. Gower, M.S., and John van de Erve, Sr., M.D.,
Charleston, S. C.

THE slow intra-arterial injection of one-sixth molar solution of sodium carbonate during the course of blood pressure experiments has been found very satisfactory for preventing clotting in the arterial cannula. In the Trendelenburg apparatus heretofore used the slow injection is accomplished by allowing mercury to drip slowly from a burette or mercury bulb into a stoppered flask which contains the solution and is connected with the manometer system (Fig. 1). The entrance of mercury into the closed system gradually displaces the fluid column in the tubing toward the cannula tip and the mercury flow is adjusted to keep the blood and anticoagulant mixing at that point. The same principle of fluid displacement by mercury has been used for the slow-timed intravenous injection of drugs.[1]

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For general use in student laboratories the method has been found impractical because of the high cost of adequate amounts of mercury and the frequency of accidents with it in the hands of students. The simple arrangement described below eliminates the use of mercury but retains the advantages of the injection method.

In the apparatus, as diagrammed in Fig. 2, a source of compressed air is used to force the solution from bottle *B* into bottle *A*. The rate at which the solution enters bottle *A* is regulated by adjusting the screw clamp *E* on the connecting tubing, and is observed by watching the rate of drop formation from the inlet tube in the air space at the top of bottle *A*. As only solution enters *A* the size of the air space remains constant except for slight changes with pressure fluctuations. Solution entering *A* raises the pressure and causes displacement of fluid from *A* into the manometer system.

The influence of the compressibility of the air in the air cap of bottle *A* on the movement of fluid in the manometer system can be eliminated by

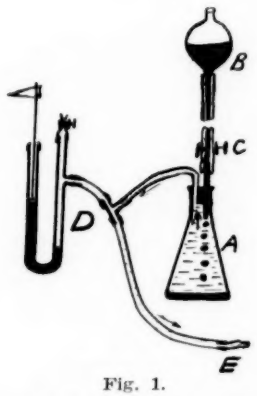

Fig. 1.

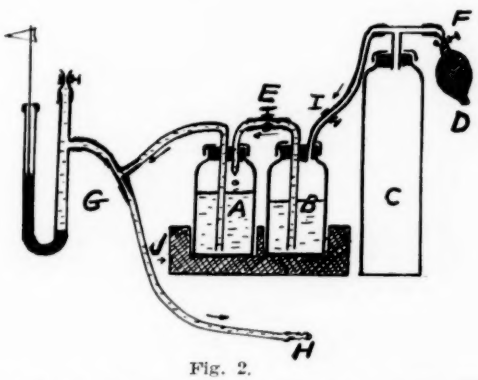

Fig. 2.

Fig. 1.—*A*. Flask containing M/6 Na_2CO_3. *B*. Bulb containing mercury. *C*. Screw clamp for regulating the rate at which mercury enters the flask. *D*. Manometer system consisting of manometer and tubing. *E*. Arterial cannula.

Fig. 2.—*A* and *B*. Pressure bottles fitted with rubber stoppers, which are held in place by screw caps. *C*. Any source of compressed air. A bottle as represented may be filled with a hand bulb (*D*) after which the screw clamp *F* is closed. *E*. Screw clamp regulating the flow of fluid from *B* to *A*. The rate of flow is observed by watching the drops fall through the air cap in *A*. *G*. Manometer system. *H*. Arterial cannula. *I*. Screw clamp. *J*. Wooden base for mounting bottles.

reducing the quantity of air to a minimum and by interposing a degree of relative resistance between *A* and the manometer system. Details for accomplishing this are shown in Fig. 3, in which the inlet tube (*A*) is connected by means of rubber tubing with a burette funnel (*C*), through which a small glass tube (*D*), drawn to a fine point, is inserted to make a water-tight connection with the tubing about the funnel. With this addition air is forced from the tubing into the funnel, followed by the fluid from bottle (*B*). The excess of air escapes to the top of the bottle and passes out through a special outlet tube governed by a screw clamp (*G*). The tip of the outlet tube (*F*) is fire polished to give a pinhole aperture.

If desired a simple manometer may be added to bottle *B* to indicate changes in the air pressure available. This is especially desirable when compressed air in a bottle is used as the source of pressure. A simple design

made from a heavy glass pipette is shown in the detail drawing of bottle *B*. (Fig. 4.) Bottle *B* may be conveniently graduated to indicate the total amount of fluid injected.

As pressure conditions exist in both bottles *A* and *B* some means must be devised to keep the stoppers in place. We have found it convenient to use square histologic specimen bottles with a screw cap for both *A* and *B*. The ones in use have a capacity of 140 c.c. The wide mouth takes a No. 6 stopper, which is cut off to rest flush with the glass when in place. The center of the screw cap is cut away to just clear the holes in the stopper. When in place the screw cap overlaps the stopper at the margin and prevents it from being forced up by the pressure.

If direct connection with a pressure line is not available a liter bottle filled with a hand bulb, or better, a tire pump is a very satisfactory source of

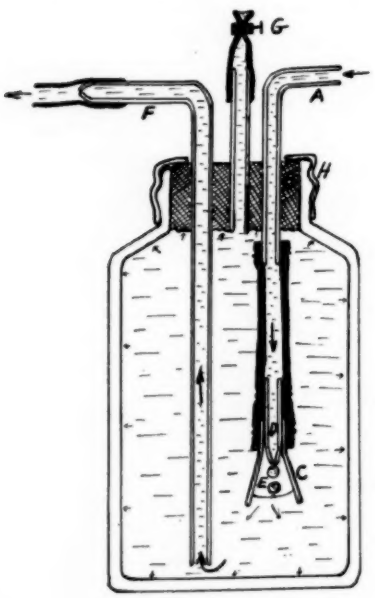

Fig. 3.

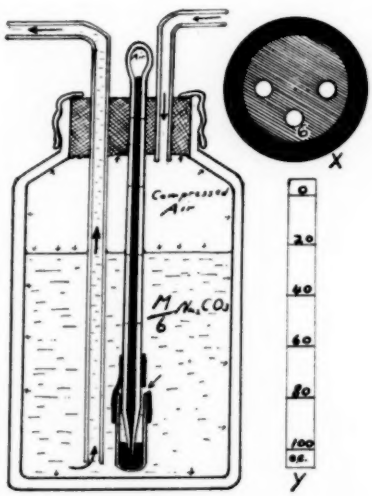

Fig. 4.

Fig. 3.—Modification of bottle *A* of Fig. 2. *A*. Inlet tube; *B*. Stopper held in place by the screw cap *H*; *C*. Burette funnel; *D*. Small glass tube with dropper point. *E*. Air space in which the drops are observed. *F*. Outlet tube with tip fire polished to give a pinhole opening.

Fig. 4.—Bottle *B* in Fig. 2 with pressure gauge. A segment of a fine bore pipette is sealed in a flame at one end and the end expanded by air pressure while hot. The other end is held by the friction of a rubber connection in a small vessel containing mercury. The latter is made by annealing one end of a piece of glass tube in the flame until closed. An opening in the rubber is made as indicated to transmit the pressure to the mercury. *X*. Top view of stopper and screw cap. *Y*. Calibration of bottle.

pressure. (*C* in Fig. 2.) One filling with reasonable pressure is more than sufficient to carry on a prolonged experiment displacing contents of bottle *B* several times.

For convenient handling, bottles *A* and *B* are mounted together in a block of wood in which snugly fitting sockets have been cut.

To use the apparatus, bottles *A* and *B* are filled to the desired level from a stock bottle by connecting a siphon tube with the glass delivery tube extending to the bottom of the bottles. The displaced air escapes from the other

opening. The apparatus is connected as described. With the pinchcock between *A* and *B* closed, air pressure is admitted to *B*. The pinchcock between *A* and *B* is then gradually opened, the air displaced from the tubes of the manometer system, the cannula tied in the artery, and pressure in the manometer raised to approximate the blood pressure expected. The bulldog clamp, guarding the artery, is then removed and the fluid column allowed to oscillate with blood pressure fluctuations. Fluid is admitted into *A* at a rate which just keeps the anticoagulant and the blood mixing in the cannula tip. When bottle *B* is empty, the record of blood pressure need not be interrupted. The screw clamp between *A* and *B* is tightened, the air supply is shut off, air is allowed to escape from the inlet of bottle *B*, and the bottle then disconnected and filled as before. The bottle is then reconnected, the pressure restored, and the rate of admission into bottle *A* adjusted at the desired rate.

By substituting a burette for bottle *B* and supplying air under pressure to the water column of the burette, the principle described is applicable to the slow injection of drugs under pressure at a measured rate. For more rapid, and less accurately quantitative, injections the calibration of bottle *B* may suffice. In either case the outlet tube from bottle *A* is connected by tubing directly with the needle used for the injection.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CISTERN PUNCTURE: Jacobi, L. Arch. Dermat. & Syph. **19**: 651, 1929.

The author favors the method described below:

The patient may be sitting up or lying on one side; each position has its advantages and its drawbacks. The upright attitude offers clearer landmarks, but it requires an assistant to hold the head flexed and the use of a syringe to aspirate the fluid, as there is usually no flow after a successful puncture, owing to negative pressure on the cistern. The recumbent position allows one to dispense with assistance and aspiration, the fluid flowing freely because of the positive pressure inside the punctured cavity. In spite of the somewhat greater difficulty of keeping the instrument in the median plane, the consensus of expert opinion favors the horizontal position.

Scrupulous asepsis of the needle, the hands and the field of operation is, of course, the first indispensable condition. Painting the nape of the neck with tincture of iodine is sufficient. The stain may afterward be removed with ammonia water or a solution of sodium thiosulphate.

The patient lies on the right side with the head moderately flexed and resting on a small hard pillow, which must be high enough to impart to the cervical spine a perfectly horizontal position. Moreover, the head itself should lie at right angles to the spine, or, as some express it, the cervical vertebrae, and the external occipital protuberance should form one straight line. Attention to these seemingly pedantic details may mean the difference between success and failure, since any considerable deviation from the "square" position will impart a wrong bias to the needle. Extreme flexion of the head is likewise to be avoided, as it tends to efface the osseous landmarks, though some degree of inclination is needed in order to put the atlanto-occipital membrane on the stretch.

With these preliminary requirements fulfilled, the operator is ready to proceed to the actual puncture. Placing the left index finger on the external occipital protuberance, he palpates downward in the middle line, closely hugging the occiput, until the fingertip reaches the deepest depression. Immediately behind this spot will usually be found the spine of the axis, which is the first essential bony landmark. About 0.5 cm. above it, the needle is inserted, pointing in the approximate direction of the outer end of the eyebrow (Spiegle).

No exact instructions can be given in this particular, as the proper direction of the needle will vary considerably with the degree of flexion of the head. For example, if the head is bent very slightly, the needle will have to be directed toward the eyebrow; on the other hand, with the head in extreme flexion and the chin resting on the chest, the needle will point proportionately higher. It will thus be readily seen that the imaginary prolongation of the instrument must describe an arc of several centimeters, corresponding to the degree of inclination of the head.

For this reason, Eskuchen himself does not specify any external landmarks for directing the needle, beyond the mere statement that it should be aimed at the place where one expects to find the end of the occiput. Wartenberg tries to be more explicit by giving the position of the instrument at the end of the operation. According to his observations, an imaginary prolongation of the needle, after it has reached the cistern, will pass between the eyebrows, or from 2 to 3 cm. higher. He advises the operator to bear this terminal position in mind at the beginning of the puncture, and to be guided by it in choosing the right direction. When in doubt, he thinks it is better to aim a little higher, in order not to fall short of the occiput. Eskuchen also prefers to "reach the bone too soon rather than miss it altogether."

To the novice all these instructions may seem confusing, but experience will soon enable him to find his way without difficulty.

Puncture of the skin often meets with considerable resistance and should be made with a quick thrust, at the same time "holding back" in order to avoid a sudden plunge forward. After passing the skin, the needle, advancing strictly in the middle line and in the direction just detailed, will soon encounter a bony obstacle, which is the squamous portion of the occiput.

The objective of the needle is the posterior edge of the foramen magnum. This second and extremely important bony landmark is called the point of orientation, because it serves as a guidepost for the next and final phase in the entire procedure, namely, the perforation of the fibrous atlanto-occipital membrane, which is all that now intervenes between the needle and the fluid in the cistern. Not always can this point on the edge of the foramen be reached at the first attempt. More frequently the instrument will impinge on the bony area above the edge, and it then becomes necessary to feel one's way along the occiput toward the foramen, by tapping the bone lightly with the needle, after the manner of a blind man's staff, at each tap lifting the handle slightly and thus lowering the point. This manipulation soon brings the needle down to the foramen, and the exact place on its edge where the tapping instrument slips off the bone and meets the elastic resistance of the ligament below is the point of orientation. Here the atlanto-occipital membrane is attached to the bone, and here the conditions for obtaining fluid are most favorable, as the cistern is at its deepest about this level.

Should it happen that the needle in its first advance has fallen short of the occiput, the point is raised by lowering the handle, and the groping is continued upward until the bony edge is located.

The point of orientation is the crux of the entire Eskuchen method, and it must be felt in every instance before proceeding further.

With the needle at the point of orientation, the operator raises the handle of the instrument in order to lower its point, removes the stylet and again advances very slowly and cautiously for a distance varying from 0.5 to 1.5 cm. The latter figure must not be exceeded, for it marks the outer limit of safety. Usually, the lumen of the cistern is reached sooner, sometimes after going forward a few millimeters. As the needle enters the cavity, there is a characteristic muscular sensation of resistance suddenly giving way, and almost immediately fluid appears at the hub. Sometimes this feeling of "give" is absent; hence the wisdom of removing the stylet in order not to "overpass."

SYPHILIS: Delayed Darkfield Examination, Mahoney, J. F., and Bryant, K. K. Ven. Dis. Inf. **11:** 103, 1930.

A number of fine, straight, glass capillary pipettes about 8 cm. in length and of constant bore are prepared.

The suspected lesion is abraded gently with gauze, the resulting blood sponged away, and the serum allowed to collect on the face of the lesion. The end of the capillary tube was then touched repeatedly to the lesion and the serum allowed to flow into the tube by capillarity. A mixture of 50 per cent vaseline and paraffin was used for the purpose of sealing the tubes. Press one end of the collection tube into a vaseline-paraffin mixture until the plug at the opposite end is forced out and the serum collects in a drop at the upper end of the tube. The same objective can be accomplished by inserting a finely drawn capillary pipette into the lumen of the collection tube and aspirating the serum.

SPIROCHETA PALLIDA: Staining of (Third Communication), Yamamoto, T. Acta Dermat. Kyoto **14:** 145, 1929.

In the first communication regarding staining of spirochetes the author found that 452 stains showed up spirochetes and he singled out 200, which yielded as good, or better, results as the twenty-four-hour Giemsa stain, for the three spirochetes named in the title. Testicular

syphilis and frambesia of rabbits was used on the height of infection. Spirocheta cuniculi was taken from condylomata near the rabbit anus. The smears were treated with a solution of ether and alcohol, equal parts, for fifteen minutes. All stains were used with 1 per cent watery or saturated solution, excepting in some instances a 5 per cent carbolic acid was added as a corrosive.

The dyes used in this study were methyl violet, 1713, which produces a deep violet color in Spirocheta pallida and pallidula, while it stains Sp. cuniculi violet blue. A few of the dyes, though of the same structure, produced other colors. Other stains used were baselin blue, erythrosine, neptune blue, acid blue, with carbolic acid, union violet, acid green, alkali blue, of various combinations with carbolic acid. Acid blue BBX with carbolic acid stains Spirocheta pallida and pallidula better than the twenty-four-hour Giemsa stain. Sp. cuniculi shows up negative under this same stain. Alkali blue always stains Sp. pallidula blue and Sp. cuniculi negative.

B. TYPHOSUS: New Medium For, Focosi, M. (Sull Utilita del Terreno al Ferrociannro-Tartrato Ferrico Potassico per l' Isolomento del Bacillo del Tifo). Diagnostica e Tech. di Lab. **1**: 246, 1930.

The following solutions are required:

1. Potassium ferritartrate 40 gm.
   Distilled water q.s. to make 200 c.c.
   Dissolve in the cold and add 4 c.c. of liquid phenol.
2. Lactose 250 gm.
   Distilled water q.s. to make 600 c.c.
3. Potassium ferrocyanide 40 gm.
   Distilled water q.s. to make 200 c.c.
4. Normal saline.

The lactose and potassium cyanide are first dissolved, a small quantity of distilled water heated in a water-bath and, when made to quantity indicated, are sterilized by heating to 100° C. for thirty minutes.

The solutions are then mixed as follows:

Sol. 1:180 c.c.
Sol. 2:430 c.c.
Sol. 4: 40 c.c.
Sol. 3:175 c.c.

This stock solution may be kept indefinitely in a sterile well-stoppered container. A greenish sediment may form and should be suspended by shaking before using the solution.

For use to 10 parts of the stock solution add 1 part of melted agar and after thorough mixing, pour plates. The reaction may be between P_H 7.2 and 7.8 but should not exceed 7.8.

Either pour or streak plates may be made.

B. coli gives azure colonies due to the formation of acid by fermentation of the lactose and the consequent production of Prussian blue.

B. typhosus colonies are yellowish-white in color.

BLOOD SUGAR: Accurate Determinations with One-Tenth and Five-Hundredths Cubic Centimeters of Blood, Pickard, R. J., and Pierce, L. F. J. A. M. A. **94**: 1134, 1930.

The reagents used are tungstic acid solution, prepared by mixing 1 c.c. of 10 per cent sodium tungstate, 1 c.c. of two-thirds normal sulphuric acid and 8 c.c. of distilled water; Folin-Wu alkaline copper solution, and phosphomolybdic acid solution, which is discarded if it shows more than a pale color. Standard sugar solution containing 1 mg. of dextrose per 10 c.c. is made from 1 per cent stock dextrose solution preserved with toluene or xylene.

APPARATUS AND TECHNIC

The apparatus comprises a pipette measured to certain 0.10 and 0.05 c.c. for obtaining blood; a pipette graduated at 1.9 c.c. (0.1 c.c. blood) and at 0.95 c.c. (for 0.05 c.c. blood specimen). Serologic pipette may be used if properly graduated. Heavy-walled tubes 10 cm. long, with an inside diameter of 1 cm. are used for centrifugation. Folin-Wu sugar tubes graduated at 6.25 c.c. and at 3.125 c.c., and Ostwald Folin 0.50 and 0.25 c.c. pipettes, are employed for the measurements.

A 1.9 c.c. portion of the tungstic acid solution is pipetted into the 10 by 1 cm. tube. Into this, 0.1 c.c. of blood obtained by ear puncture is expelled and washed, and mixed by shaking; when a chocolate color appears the tube is placed in the centrifuge and precipitated at high speed. (For 0.05 c.c. of blood, 0.95 c.c. of tungstic acid solution is used.) The usual Folin-Wu technic is then followed, 0.5 c.c. of the supernatant fluid being pipetted into a Folin sugar tube with a 0.5 c.c. Ostwald pipette, 0.5 c.c. of the standard into another tube, 0.5 c.c. of the alkaline copper solution added to each and both placed in boiling water for six minutes. They are then cooled; 0.5 c.c. phosphomolybdic solution is added to both the standard and the blood; the solution is allowed to stand five minutes to develop the color; diluted to the mark, mixed, and read in the colorimeter. If the unknown is set at 20 mm. in the colorimeter and matched against the standard, the reading of the standard multiplied by ten is the sugar content in milligrams per hundred cubic centimeters of blood. For example, if the unknown is set at 20 and the standard matches at 10.2, the blood sugar is 102 mg. per hundred cubic centimeters of blood. Readings should be recorded to the nearest 5 mg.

The greater error inherent in the Folin-Wu method with high dextrose readings is the reason for the dilution of the blood to 1:20. The tungstic acid solution contains double the proportion of acid ordinarily used; with 0.10 c.c. of blood this gives a more rapid precipitation and a more concentrated precipitate by centrifugation, with a resultant larger amount of clear supernatant fluid. Rechecks can be made without difficulty on the same specimen.

MALARIA: Thick Film Method, Barber, M. A., and Komp, W. H. W. Pub. Health Rep. **44:** 2330, 1929.

The authors insist on the importance of a good quality Giemsa stain and that the water used for diluting it should be neutral or slightly alkaline and free from salts. They employ Grubler's Azur Eosin solution, which can be obtained from Karl Hollborn of Leipzig. The distilled water should have a reaction of P_H 7.0 to 7.2. The blood can be conveniently collected from a puncture made on the dorsum of the middle finger a little below the base of the nail, care being taken not to touch the patient's skin with the slide, which must be free from grease. A drop of blood, about three or four times as much as is required for a thin film, is taken onto the slide near one end, and is spread out with the pricking needle over an area the size of the little finger nail. The patient's number is written on the other end with a grease pencil. The slide is placed, film side downward, in its groove in the slide box, which is stood on its end until the blood is dry enough not to run. Slides kept overnight in a closed box are sufficiently dry for staining next morning. Alternatively, the box may be left without its lid in the incubator for sixty to seventy-five minutes. If drying is insufficient, the film will come off in the staining bath; if it is too prolonged, the parasites will not stain properly. If it is necessary to keep the slides for some time before they can be stained, excessive drying can be prevented by wrapping them in paraffined (not kerosined) paper. The authors find that preliminary dehaemoglobinization and fixation of thick films are unnecessary. Sufficient stain for 25 slides is prepared by putting 60 or 70 drops (1.3 c.c.) of Giemsa solution in the staining dish and adding 75 c.c. of water. The slides are left in the stain for about half an hour. Differentiation is obtained by placing them in distilled water for five minutes. If the background is deep blue and the leucocytes almost black, the preparation is overstained. Thin films may be made on the same slide as the thick films, at the other end, and the labeling done with an ordinary lead pencil on the thin film. The thin film can be stained later with Leishman's stain, if it is necessary to

determine the type of parasite; a line drawn across the slide with a grease pencil prevents the stain running on to the thick film. It is unsafe to call anything a parasite unless it shows a red dot of chromatin associated with a blue mass of cytoplasm. Stained films can be preserved by covering them with liquid paraffin, or vaseline, and storing away from light. Immersion-oil must first be removed with xylol, after warming the slide slightly, and the xylol removed with absolute alcohol. When large numbers of slides require staining, small pieces of cardboard can be placed between the numbered ends, and the slides held in place by a rubber band. The block of slides can then be placed upright in the staining dish, with sufficient staining solution to cover the thick films at the other end.

TISSUE: Rapid Diagnosis of Malignant Tumors, Dengler, R. Zentralbl. f. Gynäk. 53: 457, 1929.

A rice seed sized piece of tissue is removed from the suspected part and dissected with the help of two needles in 0.9 per cent NaCl solution. It is examined with high magnification and almost closed diaphragm after the addition of a drop of 1 per cent acetic acid. The nuclei of the tumor cells are counted according to their size. The smallest nuclei present are counted with 1, the nuclei twice the size with 2, etc. to 7. The result is expressed as a quotient of the number of different sizes counted divided by the total number of nuclei counted. If fifty nuclei are counted, the quotient for carcinomas is always above 0.1, usually 0.14. The range, in general, is from 0.06 to 0.14 (from 3/50 to 7/50). The diagnoses were made from fresh tissue in 100 cases and later substantiated from sections prepared with the usual methods.

B. TYPHOSUS: A New Method of Isolation from Feces, Tifico, B. Bull. d. Sez. Ital. d. Soc. Intern. di Microbiol. 1: 275, 1929.

The author adds to the fecal suspension typhoid agglutinating serum and, after standing, centrifuges.

The sediment is washed with normal saline, a new dose of agglutinating serum added and again centrifuged. It is then plated.

STREPTOCOCCI: From Scarlet Fever, Erysipelas, and Septic Sore Throat, Tunnicliff, R. J. A. M. A. 94: 1213, 1930.

Tunnicliff reports that hemolytic streptococci from typical cases of erysipelas produce a bright green on chocolate agar after from twenty-four to forty-eight hours' growth, while those from scarlet fever cause no change or occasionally a slight greening of the medium after several days' growth.

Immunologically (opsonic method), hemolytic streptococci from septic sore throat belong to neither the scarlet fever nor the erysipelas groups. They differ from scarlet fever streptococci by causing chocolate agar to become green.

On chocolate agar, typical colonies of streptococci from scarlet fever are slightly granular and conical, and from erysipelas smooth and convex, while those from septic sore throat are very rough, indented, conical, or convex, and gun-metal in color, with occasional blister-like tops.

INDICANEMIA: Value of in Cases of Renal Insufficiency, Monias, B. L., and Shapiro, P. Arch. Int. Med. 45: 573, 1930.

The following method is described:

Either oxalated plasma or serum may be used. Plasma has the advantage that one need not wait for the clot to separate from the serum, and in that it can be taken from the samples already collected for the determination of the nonprotein nitrogen, urea nitrogen and creatinine. However, it is essential to use lithium oxalate, since potassium oxalate seems to interfere with the color reaction (as it does also in the determination of uric acid).

Serum has the advantage that no special lithium oxalate bottles are required. The blood sample may be placed in any available tube.

The following reagents are required: (1) 20 per cent trichloracetic acid; (2) 5 per cent alcoholic solution of thymol; (3) concentrated fuming hydrochloric acid (clinically pure, specific gravity 1.19), containing 0.5 per cent ferric chloride; (4) chloroform (Merck's reagent, chemically pure) and (5) distilled water.

The following apparatus is required: (1) a 100 c.c. graduated cylinder, preferably with glass stopper; (2) an Erlenmeyer flask, of about a 200 c.c. capacity; and (3) a filter funnel.

Quantities may be measured either gravimetrically or volumetrically. Five grams or cubic centimeters of the separated plasma or serum are placed in an Erlenmeyer flask. An equal amount of distilled water is added. The mixture is precipitated by adding an equal quantity, i.e., 10 gm. or c.c. of the trichloracetic acid. The flask is then rotated for about two minutes. After five minutes the contents are filtered into the graduated cylinder. Through a capillary pipette, 10 drops of the thymol solution are added to the clear filtrate. Then is added an amount of the hydrochloric acid-ferric chloride solution equal to that of the filtrate obtained. The mixture is shaken well and allowed to stand for two hours. Then 5 c.c. of chloroform is added, and the mixture is shaken for two or three minutes, and allowed to stand. The chloroform dissolves out the indigo and promptly settles to the bottom.

For accurate, quantitative readings, the chloroform is pipetted off, and the intensity of its coloration is compared with an artificial or natural standard solution. A Duboscq or Autenrieth colorimeter is used for this purpose.

Preparation of the Natural Standard. Three milligrams of indican are dissolved in 30 c.c. of chloroform and placed in a graduated glass cylinder. To this solution are added 30 c.c. of a 0.5 per cent solution of ferric chloride in concentrated hydrochloric acid, and 30 capillary drops of a 5 per cent alcoholic thymol solution. The mixture is shaken well and left standing for two hours. The colored chloroform is pipetted off, and the remaining solution is shaken with another 10 c.c. of chloroform which is added to the portion previously removed. This procedure is repeated until the total amount of chloroform is 60 c.c. This 60 c.c. contains 3 mg. of indican; 10 c.c. contains 0.5 mg., or 100 c.c. contains 5 mg. By using this chloroform solution as the standard, the calculation of the unknown quantity is done as follows:

$$\frac{\text{Reading of the standard} \times 0.5}{\text{Reading of the unknown} \times 10} = \text{milligrams of indican per 100 cubic centimeters of blood.}$$

If the coloration of the standard is too intensive, further dilutions may be made with chloroform. Of course, these dilutions are taken into consideration in calculating the unknown quantity.

Preparation of the Artificial Standard. Indican cannot be obtained on the market. It is cumbersome to prepare and requires special technical skill and laboratory facilities. The necessity of preparing an artificial standard has been recognized by earlier investigators. Thus, Erick suggested a mixture of scarlet red and eriglaucin (Gruebler). For the artificial standard, the authors selected two dyes which are found in every laboratory, namely, eosin (water soluble, yellow) and gentian violet. The artificial standard consists of 6 capillary drops of a 1 per cent solution of gentian violet in water, 3 drops of a 1 per cent solution of eosin in water, 10 c.c. of 94 per cent alcohol and 30 c.c. of distilled water. This mixture corresponds in tint and intensity to the original standard of 5 mg. of indican in 100 c.c. of chloroform. From the stock solution, various dilutions may be made, water being used as a diluent. Besides being easily obtainable, this artificial standard has the advantage of not fading. The higher dilutions of the natural standard fade with time.

To facilitate the dilutions of the standards, the accompanying tables are given.

TABLE I. (STANDARD 1)

DILUTIONS OF STANDARD SOLUTIONS WITH EQUIVALENTS OF INDICAN

AMOUNT OF STOCK SOLUTION C.C.*	AMOUNT OF CHLOROFORM ADDED C.C.	EQUIVALENT OF INDICAN MG.†	INDEX OF INTENSITY OF INDICAN
10	0	0.5	
9	1	0.45	4 plus
8	2	0.40	
7	3	0.35	
6	4	0.30	3 plus
5	5	0.25	
4	6	0.20	
3	7	0.15	2 plus
2	8	0.10	

*Stock solutions of smaller amounts may be used.
†One hundred cubic centimeters of the stock solution corresponds to 5 mg. of indican.

TABLE II. (STANDARD 2)

DILUTIONS OF STANDARD SOLUTIONS WITH EQUIVALENTS OF INDICAN

AMOUNT OF STOCK SOLUTION C.C.*	AMOUNT OF CHLOROFORM ADDED C.C.	EQUIVALENT OF INDICAN MG.†	INDEX OF INTENSITY OF INDICAN
10	0	0.05	
9	1	0.045	1 plus
8	2	0.40	
7	3	0.035	
6	4	0.030	Trace
5	5	0.025	
4	6	0.020	
3	7	0.015	
2	8	0.010	Negative
1	9	0.005	

*Stock solutions of smaller amounts may be used. Solutions may be prepared from the artificial stock standard (10 c.c. or 0.5 mg. of indican) in the same way as the natural standard solutions, water being used as a diluent.
†One hundred cubic centimeters of the stock solution corresponds to 0.5 mg. of indican.

CEREBROSPINAL FLUID: New Method for Estimation of Albumin in, Arnaud, R. Bull. Soc. path. exot. 22: 337, 1929.

The method recommended by the author consists in the precipitation of albumin at ordinary temperatures by the following reagents:

Acetic acid	5.0 c.c.
Carbon tetrachloride	1.5 c.c.
Alcohol, 95 per cent	24.0 c.c.

The mixture should be well shaken and preserved in a colored and stoppered bottle. The technic consists in pouring 4 c.c. of the cerebrospinal fluid into a Sicard's tube and adding at least 1 c.c. of the reagent. At the zone of contact of the two fluids there is a milky precipitate; the tube should then be stoppered and the two fluids well mixed. The results should be read after twenty minutes in the usual way.

It is claimed that the results are comparable with those yielded by the classical procedure, that the precipitate is even more regular and consequently the reading easier, and that it saves at least four and one-half hours.

Sice and Boisseau (Bull. Soc. path. exot. 22: 679, 1929) report as follows on this method: In the case of normal spinal fluids and those containing but slight excess of albumin the differences, although marked, are not of great significance; but as the quantity of albumin increases so do the differences in values obtained.

The conclusion reached is that Arnaud's new method does not constitute any advance and should not replace the classical method of employing hot trichloracetic acid for five hours.

B. DIPHTHERIAE: The Stoltenberg Stain, Owen, H. H., and Band, M. Am. Jour. Pub. Health **20:** 426, 1930.

The formula and procedure for Stoltenberg's stain are as follows:

Malachite green	0.250	gm.
Toluidin blue	0.050	gm.
Hematoxylin or logwood extract	0.010	gm.
Distilled water	100.0	c.c.
Acetic acid	3.0	c.c.
Ethyl alcohol	3.0	c.c.

Stain for 1 minute. Then wash the preparations with tap water and dry. The granules appear red and the body green.

The stain is not recommended as a substitute for Loeffler's alkaline methylene blue stain for routine use as it does not as satisfactorily differentiate B. diphtheriae from B. xerosis, and other nonpathogenic forms with polar staining. It is recommended as a valuable confirmatory stain, however, since it aids in the differentiation of B. diphtheriae from B. hofmanni, especially in young cultures, and is a very short and simple procedure.

SPIROCHETES: Silver-Starch-Gelatine Method for Tissues, Warthin, A. S. Brit. J. Ven. Dis. **5:** 255, 1929.

A. For material well fixed shortly after removal from body.

1. Remove paraffin from cover-glass preparation, pass through alcohol and water, and place in the oven in about 10 c.c. of 1 per cent nitric acid for thirty minutes.

2. Wash in distilled water for ten to fifteen seconds, and then continue with the modified Warthin-Starry method.

B. For material poorly fixed or showing postmortem changes.

1. Remove paraffin from cover-glass preparation, pass through alcohol and water, and place in 1 per cent nitric acid for one minute.

2. Wash in distilled water for ten to fifteen seconds, and continue with the modified Warthin-Starry method.

In the case of old tissues much over-fixed in alcohol the following routine may be used:

1. Remove the paraffin from the cover-glass preparation, pass through alcohol and water, and place in nitric acid of 2 to 10 per cent strength for from fifteen to sixty minutes. The greater the acid concentration, the shorter the period of immersion in the acid necessary.

2. Wash in distilled water from fifteen seconds to three minutes. The stronger the acid used the longer should be the period of washing.

3. Dip in 2 per cent silver nitrate and proceed with the starch gelatine method. Longer development may be necessary with the increased acidity of the section, but there results a lighter background, with heavily stained organisms and a cover-glass almost free from precipitates.

This method of "freshening" the tissue has been used with great success in stock control tissue that has been kept in the laboratory for over twenty years, in which it was becoming increasingly difficult to obtain well-stained spirochetes. Hydrogen peroxide had been used with varying degrees of success, but at the best was uncertain. By the use of nitric acid as above directed beautiful preparations are now easily obtained with this old material. The spirochetes appear intensely black on an almost colorless background. Other material which had been preserved under similar conditions, but not for so long a period, gave even better results. The sections, dried on the cover-glass without the use of albumin fixative, become detached when the stronger acid solutions are used for an hour or longer at a temperature of 30° to 40° C. On the other hand, in dilutions too great the tissues are not sufficiently acted upon by the acid to produce the proper result. In fresh cases fixed for not more than several days and then embedded in paraffin the acid treatment should not be

prolonged in case the fixation was poor or the spirochete on the point of breaking up when fixed, since the action of the acid tends to break up the spirals and give the beaded effect of degenerating spirochetes. In such tissues the sections may be dipped in dilute acid for one minute, and a favorable tissue reaction thus established.

Directions for using the Starch-Gelatine Modification of the Warthin-Starry Method of Staining Spirocheta pallida in Single Tissue Sections on the Cover-Glass, with the Preliminary Nitric Acid Treatment:

1. The tissues should be well fixed in formol, larger pieces requiring more time than smaller ones.

2. Transfer blocks of convenient size to 96 per cent alcohol for one hour or longer. Follow by three changes of absolute ethyl alcohol for one hour each at a temperature 50° to 55° C.

3. Run through two changes of xylol for half an hour and an hour respectively at room temperature.

4. Press the xylol out of the tissues on filter paper and pass through two changes of paraffin for half an hour and eight to twelve hours each at 50° to 55° C.

5. Block in paraffin.

6. Cut sections 6 to 10 microns thick and transfer on to water just warm enough to flatten the section without melting the paraffin at about 35° to 40° C. Distilled water free from bacteria must be used, or there will be a precipitate of silver between the tissue and the cover-glass.

7. The perfectly clean cover-slip is then immersed in the water perpendicularly at the edge of the floating section and then lifted out with the section on it. If the glass is wholly free from oily contaminations the tissue will adhere to it. No albumin fixative is used. Number 1 cover-slips of suitable dimensions are employed, and with a little practice the sections can be readily centered.

8. The cover-glass preparations are then allowed to dry for two hours at 55° C. or overnight at 35° C.

9. The paraffin is removed from the section by flaming slightly and putting through two changes of xylol, two changes of absolute alcohol, 96 per cent alcohol and water.

10. The cover-glass preparation may now be treated with nitric acid solution as described above or immersed directly in 2 per cent silver nitrate, and placed face down upon another perfectly clean cover-slip (the tissue then being between the two cover-slips), and the two cover-glasses adherent by capillary attraction are put upright at the edge of a clean bottle containing sufficient silver nitrate solution to cover the lower half of the two cover-slips only. When nitric acid is used the section must be washed as directed in distilled water before it is put into the silver.

11. The incubation in the silver nitrate solution should be carried out in a dark oven at 50° to 55° C. for from thirty minutes to two hours, depending on the type of tissue involved. A dense fibrous and elastic section, such as aorta, requires longer impregnation than does one of liver or lymph node. Fetal tissues and those treated with nitric acid require less exposure to silver nitrate than do those of adults, especially if overfixed.

12. The opposed cover-slips with the section between them are taken from the silver solution and the cover-glass forceps slipped between them, prying them apart. It is unwise to slide the glasses apart, as the tissue may be injured or partially loosened from the cover to which it is adherent. The cover-glass with the tissue is placed section side uppermost in a watch-glass or other flat container, and the developing mixture, consisting of 1 part of 2 per cent silver nitrate added to the starch-gelatine-acetone-quinol 5 parts (see below), is poured over it, so that a layer at least 3 mm. thick is over the tissue.

The reduction is allowed to proceed until the section is of a medium yellow or pale brown appearance.

13. The section is then removed from the developer and washed for a few seconds in warm water to ensure removal of the starch and gelatine.

14. It is then passed through a 5 per cent sodium-hyposulphite solution to remove any remaining silver nitrate.

15. Wash in distilled water, 96 per cent alcohol, two changes of absolute alcohol, two changes of xylol, and mount in balsam.

DEVELOPING SOLUTION

The developing solution is made up as follows:

1. Ten grams of gelatine are dissolved in 100 c.c. of distilled water, using a double boiler to prevent burning.

Strain while hot through a clean cloth into the stock bottle.

2. Ten grams of starch (Argo or Kingsford's) are mixed with a few c.c. of cold distilled water to make a thick paste, and then 100 c.c. of boiling distilled water are added. This is stirred and then poured into the hot gelatine in the stock bottle without straining.

3. Five c.c. of acetone are dissolved with 7 c.c. of a fresh 5 per cent solution of quinol and are added to the starch and gelatine in the stock bottle, which is immediately tightly stoppered and vigorously shaken.

4. This mixture is allowed to cool, when it will become solid. Whenever it is to be used it is placed in the oven and warmed until liquid.

5. Just before the developer is poured over the section a 2 per cent silver nitrate solution is added in the proportion of 1 to 5 and well mixed by pouring from one beaker into another several times.

The fewer the sections developed at one time the better the results, but any number may be done, as long as there is sufficient of the developer to cover all to the uniform depth of 3 to 5 mm. In this laboratory never more than eight sections are developed at once.

6. If the stock developer is allowed to stand in the melted condition for very long at a time it will settle to the bottom, in which case a vigorous shake will restore it to its original condition. It is unnecessary to add any preservative to prevent bacterial decomposition.

BLOOD GROUPS: Determination of in Cadavers, Holzer, F. J. Klin. Wchnschr. **8**: 2427, 1929.

Holzer points out that the determination of the blood group in cadavers is frequently difficult because of the plasma of the blood which is not sufficiently clear to permit demonstration of the agglutinins. It is known that the agglutinins may also be found in other body fluids. In cadavers the pericardial fluid remains free from blood pigments and from putrefactive bacteria longer than other fluids; consequently it was considered the best material for the determination of the blood groups of cadavers. The author states that this method was employed in a large number of corpses, and it proved particularly valuable in determining the blood group of cadavers of infants and of fetuses.

BLOOD PARASITES: Demonstration of in Peripheral Blood of Kala-Azar, Shortt, H. E., Das, S., and Lal, C. Indian Jour. Med. Res. **15**: 529, 1927.

A small drop of blood is placed at one end of a slide, a second slide is applied to it, as in making an ordinary blood smear. The second slide, as soon as the blood has spread out along its edge, is pushed along the surface of the first with an even motion until the blood is almost exhausted. At this point, instead of continuing this motion, as in making an ordinary smear, the slide is abruptly lifted off, with the result that the blood smear ends in a straight edge stretching transversely across the slide. This straight edge is somewhat thicker than the rest of the smear and contains a large percentage of the total white cell content of the drop of blood. The white cells in the straight edge are all that it is necessary to examine for the purpose of determining, with a fair degree of accuracy, the presence and numbers of Leishman-Donovan bodies in the peripheral blood.

RETICULOCYTE COUNT: In Normal and Abnormal Conditions, Friedlander, A., and Widemeyer, C. Arch. Int. Med. 44: 210, 1929.

The following method, for which great accuracy is claimed, is used by the authors:

A specific pipette for the making of a blood dilution of 1:20, instead of the usual white cell pipette (dilution 1:10) is used.

A microscopic objective, giving a magnification higher than the ordinary 4 mm. lens, is of advantage. The authors have used a No. 7 Leitz or a DD Zeiss. The oil immersion lens cannot be used.

The solutions needed are:

1. A 1 per cent aqueous solution of brilliant cresyl blue. We have used the Gruebler, natural analine and Coleman-Bell dyes. The Coleman-Bell dye has given the best results.

2. A diluent solution containing 0.6 cm. of sodium chloride and 0.2 gm. of potassium oxalate to 100 c.c. of distilled water.

These are the stock solutions. They can be kept indefinitely in a cool place. As needed, another dilute solution is made up as follows:

Two cubic centimeters of the brilliant cresyl blue solution (solution 1) is added to 8 c.c. of the diluent solution (solution 2) which makes a 0.2 per cent solution of brilliant cresyl blue. This solution (solution 3) must be made up fresh about once a week. It is necessary to vary the dilution from time to time, depending on the dye used.

The blood is drawn up to the 0.5 mark of the pipette with a dilution of 1:20, then it is diluted to the 21 mark with the 0.2 per cent brilliant cresyl blue solution (solution 3). The pipette is shaken thoroughly, allowed to stand for ten minutes or more and then shaken well again. The blood is then ready for counting. The ordinary counting chamber is used, and the squares are counted as they are in the ordinary white cell count. In calculating the result it must be remembered that the dilution is twice that of the ordinary white cell count. The blood, diluted in this way, may stand twenty-four hours without injury to the cells, but after this period the count is not accurate. To count the reticulocytes, it is necessary to have a good light, preferably artificial, and an especially high magnification dry lens as before mentioned. With this dilution of stain, the white blood cells stain dark blue. The red cells have a pale yellow cast, but show up blue. The reticulocytes are easily recognized by the blue reticulum or granules in their cytoplasm. The various forms of reticulocytes, as enumerated by Seyfarth, are brought out distinctly. These vary from the earliest forms, with dense reticulum in the center of the cells, so that they resemble normoblasts, to the more mature forms showing only a few scattered basophilic granules.

When normoblasts or megaloblasts are present, there is a distinct difference in staining reaction between the nuclear and reticular material. The nuclear material stains purple; the reticular substance stains blue.

This method overcomes the chief objections to the Cunningham method which is the method in general use. In the Cunningham method the stain does not come in contact with the cells of the blood droplet, and the reticulocytes are not evenly distributed over the slide. It is much simpler than counting 1,000 red cells in a stained preparation. As a matter of fact, by this method a reticulocyte count can be done as easily, and almost as quickly, as a white cell count after some experience has been had with the method.

TUBERCULOSIS: A Cholesterol Agglutination Reaction, Hinton, W. A., and Stuart, G. O. N. E. J. Med. 202: 327, 1930.

The dried and killed growth of three strains of tubercle bacilli, after being kept in stoppered glass bottles for approximately four months, were treated as follows:

One part by weight of the bacillary mass is extracted with eight volumes of ether, during the extraction shaking for ten minutes. This extraction is repeated four times.

The ether-insoluble bacillary residue remaining is then extracted for three days at room temperature with five volumes of 95 per cent alcohol, shaking vigorously several times each day.

For the conduct of the test the "glycerinated indicator" is prepared each time as follows:

To 1 part of alcoholic extract ether-insoluble bacillary residue add 9 parts of 0.7 per cent alcoholic solution of cholesterol.

To 1 part of this mixture add 2 parts of 5 per cent aqueous solution of sodium chloride and immediately shake vigorously. Then add a further 12 parts of 5 per cent sodium chloride aqueous solution and again shake. Finally add 15 parts of 50 per cent neutral glycerin and again shake vigorously.

The test:

Place 0.1, 0.3, and 0.5 c.c. of serum to be tested in each of three agglutination tubes and to each add 0.5 c.c. of glycerinated indicator. Shake well and incubate at 37° C. overnight.

A positive reaction produces definite agglutination of cholesterol particles thus clearing the fluid.

RETICULOCYTES: Determination of, Holboell, S. A. Ugeskr. f. Laeger **91:** 1077, 1929.

The following technic has been used in the author's department with satisfactory results: Place in a small receptacle 25 c.c. of 1 per cent brilliant cresyl blue in a 0.9 sodium chloride solution (the pipette may be utilized which serves for the counting of white blood corpuscles). To this add 0.1 c.c. blood (taken for example with the pipette which serves for the blood sugar determination). After careful mixture of the blood and the staining fluid, the smear preparation after half a minute is ready for the cover-slip, etc.

When the specimen has been dried in the air, it is fixed for three minutes in pure absolute methyl alcohol. Under this treatment the color will disappear except in the vital-stainable substance. The methyl alcohol is poured off, and the usual Giemsa stain is applied, treating the specimen with a freshly prepared dilution of the Giemsa solution for Romanowsky stains (Gruebler and Co.); 15 drops per 10 c.c. of distilled water. After fifteen minutes it is washed with distilled water and the specimen is dried with several layers of filter paper. Specimens prepared in this manner extremely rapidly show the existence of reticulocytes. The vital-stained substance is colored blue and differs sharply from the pale red fundamental color of the red blood corpuscles.

UREMIA: The Diazo Color Reaction In, Rubinowitch, I. M. Arch. Int. Med. **45:** 282, 1930.

A positive diazo-color reaction is not found in any condition other than severe kidney damage.

A positive reaction may frequently be found in persons with severe kidney damage, and such persons may recover. Such cases include the albuminurias of severe infections, the well-recognized form of acute nephritis with pallor, edema, etc., acute exacerbations of chronic nephritis, mechanical obstruction to the urinary flow, surgical lesion of the kidneys with urinary retention and the anuria of diabetic coma.

When acute exacerbations of the disease are excluded, a positive reaction occurring during the course of chronic nephritis has invariably, at least in our experience, meant unfavorable prognosis.

When all of the aforementioned factors are given consideration, the test is of value in differentiating uremia from cerebral arteriosclerosis.

BACTERIOLOGY: Detection of Ammonia Production in Agar Slants, Hansen, P. A. J. Bacteriol. **19:** 223, 1930.

1 c.c. of a solution of thymol and 1 c.c. of a hypobromite solution are added successively to the culture and allowed to act for twenty minutes. If ammonia is present, the mixture becomes blue or greenish blue. The blue color may be extracted by means of ether in which it is soluble, resulting in a deep-red-violet color. The reaction is also given by certain aliphatic amines and by glycine. It has the advantage over the Thomas test of not

using a hypochlorite. The hypobromite is more easily prepared fresh and of definite strength each time it is used. This is done by mixing bromine water with sodium hydroxide. Like the Thomas test, only weak solutions give the ammonia reaction.

SPINAL FLUID: Bicolored Guaiac Test, Latham, O. Med. J. Australia 802, Nov. 30, 1929.

The reagents:

1. British Pharmacopoeia tincture of guaiacum resin, 20 per cent in alcohol; a good sample freshly made up can be got from any chemist.

2. Brilliant (diamant) fuchsin, 0.5 per cent in absolute alcohol.

3. Naphthol green, 0.5 per cent in distilled water.

4. 10 per cent sodium chloride from which we make the 0.2 per cent saline used in the spinal fluid dilution.

5. A 0.5 per cent solution of anhydrous sodium carbonate.

Keep all in dark glass stoppered bottles in a cool place.

Preparing the colloidal solution:

Place 40 c.c. of distilled water in a small Erlenmeyer flask, then pour in slowly down side of flask with pipette, 0.22 c.c. of guaiac tincture in 9 c.c. of absolute alcohol (9.22 c.c. added), lightly and continuously shaking the flask. The suspension should be yellowish-white, transparent and only feebly opaque to overhead light. Still agitating slowly in a circular manner add 2 c.c. of naphthol green solution. As soon as the mixing is completed add 0.3 c.c. of the diamant fuchsin solution. The result should be a cherry red, transparent, slightly opaque mixture to overhead light. Some samples appear purplish to direct light, but seem to act. After twenty minutes it is ready for use.

The test: The usual series of dilutions are made in ten small tubes. With a pipette ½ c.c. of spinal fluid is placed in the first two tubes. Then starting from the second tube, add to each tube ½ c.c. of 0.2 per cent saline, containing 1 c.c. of 0.5 per cent sodium carbonate to each 100 c.c. of saline. The saline mixture should be made fresh from the strong saline solution. Beginning from the second tube, transfer ½ c.c. to the third and after mixing ½ c.c. to the fourth and so on, as is usual with the gold method. To each tube then add ½ c.c. of the colloidal suspension. Gently shake each tube.

Results: No change—0, the solution remains unchanged, red, without precipitate; 1, a light red color with slight precipitate; 2, greyish-red color with evident sedimentation; 3, greyish-green color, red precipitate; 4, vivid green color with red precipitate in bottom of tube. Read after twenty to twenty-four hours. A typical paretic curve would therefore read 4443321000 as in the gold solution test.

ANEROBES: A Simple Method for Petri Dish Cultures, Herrington, B. L., and Knaysi, G. J. Bacteriol. **19:** 101, 1930.

The petri dishes are poured as for aerobic cultures, except that a small amount of agar, not exceeding 8 c.c., is used. The plates are allowed to harden and are then inverted in a vacuum desiccator over a dish containing water at about 45° C. The water may be placed directly at the bottom of the desiccator. It is desirable that the desiccator walls and cover be warmed gently to about 45° C. The desiccator is then covered, connected with a suction pump and evacuated slowly until the water boils, and its vapor sweeps away the last traces of air. Just before boiling ceases, the desiccator is closed and incubated at the desired temperature for the desired length of time. The rate of evacuation should be such that thirty minutes or more are required for completion.

The method is, therefore, a combination of evacuation and the use of water vapor to sweep away the air which has, as compared with the use of the so-called inert gases, the advantages that it is the same substance as that in which the bacteria are living and that no additional expense or special equipments are required. It offers for the growth of bacteria an ideal atmosphere saturated with water vapor which, at the same time, prevents the drying up of the agar, no matter how long the period of incubation may be.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Nutrition and Diet in Health and Disease**

THIS is a book which the physician in general should welcome with enthusiasm, for if there is anything concerned with the practical application of dietetic principles in health or disease which he will not find discussed, its omission will be without significance.

With the help of this book the frequent inquiry, "what may the patient have to eat?" can be answered with specific detail and not in the general terms so often meaningless to the family.

The book is divided into three parts.

Part I (280 pages) discusses completely in a thoroughly understandable way the need for food and its utilization, food products, and diet in health, including an excellent discussion of the feeding of infants, children of school age, and adults.

Part II (295 pages) takes up nutrition in disease from the standpoint of diseases in which diet is of paramount importance and diseases in which diet is of varying importance. In all instances the directions are detailed, clear and specific including numerous menus and recipes.

There is an excellent chapter in this section on diet in obesity and leanness which is well worth studying in these days of indiscriminate enthusiasm for reducing.

Part III (87 pages) consists of tables and charts of a general nature.

There are few books on diet which should prove more useful than this to the practitioner or the dietitian. It can be highly recommended.

Clinical Atlas of Blood Diseases†

THIS volume, while small, contains a large amount of material of great value to the physician.

The authors have attempted to produce the essential features of a textbook of hematology as well as an atlas showing the striking features of blood dyscrasias.

It is not intended, of course, to be encyclopedic but rather to present in succinct form information applicable to the recognition and differentiation of the more common hematologic findings in disease.

The book begins with a glossary of the terms encountered throughout the book. Then follow five colored plates concerned with the normal and abnormal blood cells and their development. Nuclear indexes are then touched upon briefly, the remainder of the book being devoted to various diseases in which blood changes are encountered, marked, or significant.

The colored plates are well done and excellently reproduced.

The book should be well received by the physician and student and will undoubtedly be used as a reference.

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*Nutrition and Diet in Health and Disease. By J. S. McLester, M.D., Professor of Medicine, University of Alabama. Cloth, 783 pages, numerous tables. W. B. Saunders Co., Philadelphia, Pa.

†Clinical Atlas of Blood Diseases. By A. Piney, Research Pathologist, Cancer Hospital, London, and S. Wyard, Physician, Bolingbroke Hospital, London. Cloth, 99 pages, 36 illustrations of which 32 are in color. P. Blakiston's Son & Co., Inc., Philadelphia, Pa.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

*Determinative Bacteriology**

THE Lehmann and Neumann Atlas of Bacteriology with its wealth of invaluable and unique colored plates needs no introduction to the laboratory worker.

This new and extensively revised seventh edition, excellently translated by Dr. Breed, will undoubtedly receive a warm welcome not only from those to whom the work is new, but also from those who have long used the previous editions.

In this first volume, as before, are presented a large number of colored illustrations of bacteria as well as a brief but comprehensive section on technical methods.

One can only hope that Volume II will not be long in following its predecessor.

The Penicillia†

DESPITE the fact that the penicillia on the one hand are important agents for the ripening of cheese, and on the other, frequent sources of spoilage in fruits and various foodstuffs, attack fibers, wood and paper, contaminate cultures in the bacteriologic laboratory, and at times are etiologic agents of disease in the human being, very little systematic or detailed information concerning their characteristics and activities is available.

One cannot, at first, appreciate the incalculable labor entailed in the accumulation and classification of the information which has made this book possible. It represents the work of twenty years.

The volume deserves and doubtless will receive a warm welcome from mycologists as well as all those in whose field penicillia are encountered, because of the tremendous amount of information it contains concerning this species. As far as is at present possible it furnishes for the genus penicillia information analogous to that found in determinative bacteriologic manuals.

The Nursing of Infectious Diseases‡

A VERY well written small volume which physicians can safely recommend to nurses who are attending cases of contagious disease. After a general discussion of infection, the prevention of contagion, and the general management of infectious diseases, the following specific infections are discussed, scarlet fever, diphtheria, measles, German measles, smallpox, chickenpox, typhoid fever, whooping cough, meningitis, poliomyelitis, epidemic encephalitis, tuberculosis, and the venereal diseases.

Those forms of treatment have been discussed which are usually left to the nurse to carry out, or for which she must make special preparations.

Nerve Tracts of the Brain and Spinal Cord§

A COMBINED anatomy, physiology, and organic pathology of the tracts of the central nervous system. Profusely illustrated. This volume differs from the few outstanding works on central nervous system anatomy, in that it contains much on applied anatomy or neurology, and should be of distinct value as a reference manual in neurologic diagnosis. The illustrations, exemplifying attitudes, postures, and movements in the different organic neurologic diseases are especially useful.

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*Determinative Bacteriology, Vol. I: Atlas. Prof. Dr. K. B. Lehmann, and Prof. Dr. R. O. Neumann, Ed. 7, English translation by Dr. R. S. Breed. Cloth. 65 pages of colored plates. G. H. Stechert & Co. (Alfred Hafner), New York.

†The Penicillia. By Charles Thom, Mycologist, Bureau of Chemistry and Soils, Cloth, 643 pages, 99 figures. Williams & Wilkins, Baltimore, Md.

‡Lectures Upon the Nursing of Infectious Diseases. By F. J. Woollcott, M.A., M.D., B.Ch.(Oxon.), D.P.H. (Senior Assistant Medical Officer, Grove Hospital, Metropolitan Asylums Board.) Revised and Enlarged by Dorothy C. Hare, C.B.E., M.D., M.R.C.P., D.P.H. Assistant Physician, Royal Free Hospital, Examiner in Medicine for State Examination Under the General Nursing Council. Cloth, pages 195. C. P. Putnam's Sons, New York, 1930.

§Nerve Tracts of the Brain and Cord. Anatomy: Physiology: Applied Neurology. By William Keiller, F.R.C.S., Professor of Anatomy and Applied Anatomy, University of Texas. Cloth, pages 456. New York, The Macmillan Company, 1927.

*The Autonomic Nervous System**

THE advent of surgery in the domain of the autonomic nervous system has of recent years produced increased clinical interest. The volume under review is an authoritative compilation of what we know about the autonomic system today. Two-thirds of it are taken up with anatomy and physiology and the remainder with its pathology and clinical study and treatment.

The term, *involuntary nervous system* was first applied nearly two centuries ago, but this term is inappropriate because many of the reactions of the so-called voluntary nerves are not subject to voluntary control and because the nerves supplying certain of the visceral organs are not wholly free from voluntary influences.

The term *sympathetic* was introduced in 1732, by Winslow who believed that a sympathetic nerve system coordinated the functions of the different organs and that the sympathies of the body were brought about through these nerves. Another term, *ganglionic nerves* suggested by Johnstone in 1764 has not lasted.

Fichat proposed the term *vegetative nervous system* in 1800 because he believed that the life of the organism was made up of two types of life, animal life and organic vegetative life.

Langley in 1898 proposed the term *autonomic*, although realizing that this term suggested a much greater degree of independence of the central nervous system than in fact exists.

There is an excellent critical discussion of the results of sympathectomy and ganglionectomy operations as they are employed in various diseases today.

Outlines of Pathology†

WHILE this is a textbook on pathology, it is in no sense the usual dry classification and morphologic and structural discussion. The author has endeavored with great success to make his pathology a living subject. Indeed he seems to have taken as his thesis the concept that the pathologist instead of dealing with death and with dead structures is dealing with life and living structures, that diseases are physical processes of the body, and as much a part of life as health.

It is a rare experience to pick up a textbook of pathology and feel impelled to read on and on. This is such a work. We might well describe it as a pathology written by a philosopher. It should be most widely read.

Endocrine Disorders‡

A SMALL practical monograph on the clinical study and treatment of endocrine diseases. While it touches on disease of the various organs, its greatest value to the clinician will be in its discussion of the growth dystrophies. The average man of intelligence has no difficulty in recognizing obesity but is often a bit at sea as to whether it is of pituitary or gonadal origin or due to other endocrine derangement. The volume under review will aid in classification of this type of condition and offers helpful therapeutic suggestions in so far as therapy in this field is of value.

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*The Autonomic Nervous System. By Albert Kuntz, Ph.D., M.D. Professor of Anatomy in St. Louis University School of Medicine. Illustrated with 70 Engravings. Cloth, pages 576. Lea & Febiger. Philadelphia. 1929.

†Outlines of Pathology in its Historical, Philosophical, and Scientific Foundations. A Guide for Students and Practitioners of Medicine. By Horst Oertel. Strathcona Professor of Pathology, McGill University, Montreal, Canada. Cloth, pages 479. Montreal Renouf Publishing Co. McGill College Avenue.

‡Endocrine Disorders. By Professor Hans Curschmann. Director of the Medical Clinic, University of Rostock, I.M. With an introduction by Franz Prange, Doctor of Medicine and Philosophy; Assistant at the Medical Clinic. University of Rostock I.M. Humphrey Milford. Cloth, pages 188. Oxford University Press. American Branch. New York. 1929.

*Clinical Physiology**

UNDERGRADUATE physiology stands as a preparation for our understanding of clinical medicine. Too often, when we have completed the latter we have forgotten much of the details of the former. The volume by McDowall is offered we may say as an advanced physiology, presupposing an understanding of clinical medicine. It is in essence an applied physiology, the physiology of disease. It covers somewhat the same lines as that followed in Hewlett's Functional Pathology.

Diagnostic Methods and Interpretations in Internal Medicine†

THIS is, in essence, a work on physical diagnosis and the interpretation of laboratory findings. Considerable space is devoted to the technic of physical examination and special manipulations such as lumbar puncture and to the interpretation of observations. A small section is devoted to the form of the periodic health examination. The volume is quite complete and abundantly illustrated.

The Conquest of Cancer§

THIS volume may be divided into two general subdivisions, first a discussion of the nature and cause of cancer, its pathology, the manner in which it affects different organs, its treatment by methods other than radium, and, second, the radium treatment of cancer. Some space is also devoted to the use of radium in nonmalignant diseases.

The author believes that surgery is of benefit in about 50 per cent of cancer cases, x-ray in 10 per cent, and radium either alone or in conjunction with surgery or x-ray in a very much higher proportion. In advanced cases where it is a matter of relieving pain, lengthening life and minimizing the disagreeable symptoms, x-ray assumes an importance equal to surgery and radium.

He recommends surgery in cancer of the alimentary tract, except the rectum, mouth, throat, and esophagus, cancer of the central nervous system, bones, the breast, the body of the uterus. In cancer of these organs, however, he believes that operation should be preceded by the use of x-ray or radium.

X-ray is recommended in conjunction with radium in rapidly growing sarcoma and in conjunction with surgery and radium in cancer of the breast.

Radium alone is preferred in all cancers of external parts, the cervix, the bladder, and malignancy of the mouth, throat, and esophagus and rectum and in sarcomas and uterine fibroids. The latter group comprises about 40 per cent of all neoplastic growths.

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*Clinical Physiology (A Symptom Analysis). In Relation to Modern Diagnosis and Treatment. A Text for Practitioners and Senior Students of Medicine. By Robert John Stewart McDowall, D.Sc., M.D., F.R.C.P.(Edin.), Professor of Physiology, King's College, University of London. With an Introduction by W. D. Halliburton, LL.D., F.R.C.P., F.R.S. Emeritus Professor of Physiology, King's College, University of London. Cloth, pages 383. New York, D. Appleton and Company. 1927.

†Diagnostic Methods and Interpretations in Internal Medicine. By Samuel A. Loewenberg, M.D., F.A.C.P. Assistant Professor of Clinical Medicine, Jefferson Medical College; Assistant Physician to the Jefferson Hospital; Visiting Physician to the Philadelphia General Hospital; The Northern Liberties Hospital and the Eagleville Sanatorium for Caonsumptives; formerly Assistant Professor of Physical Diagnosis at the Medico-Chirurgical College and the University of Pennsylvania, Philadelphia. With 547 Illustrations, some in Colors. Cloth, pages 1032. Philadelphia. F. A. Davis Company, Publishers, 1929.

§The Conquest of Cancer by Radium and Other Methods. By Daniel Thomas Quigley, M.D., F.A.C.S. Instructor in Surgery in the University of Nebraska College of Medicine; Fellow of the American Medical Association; Member of the American Association for the Advancement of Science, Nebraska Academy of Science, North American Radiological Society, American Radium Society; Fellow of the American College of Radiology, etc.; Director of the Radium Hospital of Omaha. Illustrated with 334 Engravings. Pages 539, cloth. Philadelphia. F. A. Davis Company, Publishers. 1929.

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EDITORIALS

The Sugar Content of the Blood

FOR more than fifteen years we have possessed methods for the estimation of blood sugar which have been adequate for practically all clinical purposes. At present the choice of satisfactory methods available to the clinic is very large. Although these methods depend upon a variety of analytical principles, colorimetric, volumetric, gravimetric, gasometric and polariscopic methods having been used, most of them have been built around the reducing action of glucose, in particular upon copper reagents. It is of interest in this connection to note that as far back as the time of Claude Bernard a violent discussion arose as to the reliability of copper reduction tests in the estimation of the sugar of the blood.[1]

With the introduction of our present blood-sugar methods, most of which are sufficiently simple to permit their use in the clinic, it was generally conceded that the results obtained were probably slightly too high due to the interference of other reducing substances in the blood. It was quite justly felt that it mattered little whether the normal blood sugar was actually 100

mg. per 100 c.c. or only 80 mg., as long as the method gave consistent results. It was argued that the greater part of the reduction must be dependent upon glucose, and this was unquestionably the chief variable. Furthermore in diabetes, the clinical condition in which the determination was most used, a rise of 50 to 200 per cent was generally encountered and might reach 1000 per cent. Obviously the reducing nonsugars could not have an appreciable influence on such changes.

Slight hyperglycemias may be observed in other conditions notably in hyperthyroidism and nephritis. Some workers have questioned the reliability of the somewhat higher findings in nephritis, ascribing the increased reduction to the influence of the retained nitrogenous waste products. While some of the bodies, notably creatinine, may influence the results obtained, they do not by any means account for the hyperglycemia observed, and furthermore, quite marked fluctuations in blood sugar may take place with little change in the known nitrogenous constituents.[2] Recently Lyttle and Hearn[3] have compared the Folin-Wu and Benedict (copper I) methods on bloods showing nitrogen retention and found that the nonprotein nitrogen of the blood bore no relation to differences in the sugar content observed by the two methods. It may further be noted that the carbohydrate tolerance is decreased in both nephritis and hyperthyroidism, and the blood-sugar tolerance curves obtained resemble those found in mild diabetes.

It has long been recognized that in hypoendocrine conditions, the pancreas excepted, comparatively low blood sugars are encountered. Hypoglycemias have been observed in myxedema, cretinism, Addison's disease, pituitary disease and also in some diseases of the liver, such as acute yellow atrophy and certain forms of cirrhosis. Very low blood-sugar values may be encountered after overdoses of insulin. Do the convulsions and other symptoms occur when there is an appreciable amount of glucose in the blood, or only when the supply of glucose is completely exhausted? It is obvious that such questions can only be answered when data are available for the true glucose content of the blood.

The name of S. R. Benedict has been linked with every great advance in our sugar methods during the past twenty years. In 1909 he introduced his single qualitative reagent, now almost universally employed as a qualitative reagent for sugar in urine, and this was followed two years later by his quantitative reagent. In collaboration with Lewis[4] he introduced our first colorimetric method of blood-sugar estimation in 1913, final publication of the method appearing in 1915. Some years later, realizing that the various blood-sugar reagents were apparently reduced by substances other than glucose, he[5] attempted to prepare a copper reagent which would be more specific for glucose and in 1925 described a reagent which when applied to the Folin-Wu tungstic acid filtrate, gave results definitely lower than those obtained by the Folin-Wu method.

Folin[6] admitted that the lower results obtained by Benedict were probably nearer the true values for glucose, but objected to some of the chemical principles underlying Benedict's new method. He suggested a reagent which he thought preferable but one that gave essentially the same results. At about

the same time Harned[7] applied the Folin-Wu method to blood which had been precipitated by a mercuric reagent, which removes practically all the nitrogen, and obtained values in essential agreement with Benedict's new copper method.

Almost simultaneously with the publication of Benedict's copper Method I, Van Slyke and his coworkers[8] made an important contribution by showing that the residue of reducing substances after yeast fermentation amounted to 10 to 30 mg. per cent in terms of glucose. The use of yeast in the determination of the nonsugar reducing substances in blood has been employed to advantage by Folin and Svedberg, Somogyi, and Benedict. Folin and Svedberg[9] found that with the new Folin method and nonfermentable reducing substance amounted to 5 or 6 mg. per 100 c.c., whereas with the Folin-Wu method it was larger (about 22 mg., judging from their data). Somogyi has published several papers dealing with the nonsugar reducing substances of the blood, following yeast fermentation. With Kramer,[10] he found that the reducing nonsugar, as determined by the Shaffer-Hartmann method, amounted to about 22 mg., and that the determination of the true sugar by three different methods yielded essentially identical values. Somogyi[11] further observed that more than 75 per cent of the nonsugar reducing substances was in the cells, the average values on 36 bloods being 8 mg. for the serum and 40 mg. for the corpuscles.

Apparently feeling that his copper Reagent I fell just short of measuring the glucose content of the blood, Benedict[12] elaborated another copper Reagent II which he believes gives very closely the true glucose content of the blood when applied to the Folin-Wu tungstic acid blood filtrate. As a result of fermentation experiments and observations with this new method, Benedict concludes that the Folin-Wu technic yields figures for the blood sugar which average about 22 mg. per 100 c.c. of blood too high.

It is a well-known fact that the Hagedorn-Jensen[13] method, so much used in Europe, gives somewhat lower results than most of the methods employed in this country. This method employs a zinc salt in the precipitation of the protein. Somogyi[14] has carried out blood-sugar determinations on the blood filtrates after a similar zinc precipitation and obtained essentially the same results with four different methods on 35 separate blood samples, indicating that the zinc precipitation removes the nonsugar reducing substances. The methods used were the Shaffer-Hartmann (modified), Folin-Wu, Folin, and Benedict II.

From the foregoing it is apparent that the older blood-sugar methods, such as the Folin-Wu and Shaffer-Hartmann give results which are approximately 22 mg. too high, while the Lewis-Benedict (and the Myers-Bailey, and Benedict modifications) yield figures which are probably slightly higher.

Now that it appears to be definitely established that normal blood contains about 22 mg. of nonsugar reducing substance per 100 c.c. of blood, one naturally raises the question as to the nature of this reducing substance or substances. From the work of Somogyi it would appear that more than 75 per cent of the reducing nonsugar is present in the corpuscles. In the same issue of the *Journal of Biological Chemistry*, Benedict and Newton[15] have re-

ported observations in harmony with this finding, which go a long way toward explaining the 22 mg. of nonsugar reducing substance obtained with the Folin-Wu method. They are of the opinion that the 50 to 100 mg. of glutathione presumably present in 100 c.c. of blood exercise a large part of this reducing power and have presented some data in substantiation of this. They do not believe that the thioneine present ordinarily accounts for more than 1 or 2 mg. of reduction per 100 c.c. of blood, although occasionally it might amount to 4 to 5 mg.

It would appear therefore that the very perplexing problem as to what was the true sugar (glucose) content of the blood, which has been a topic of discussion from the time of Claude Bernard, had practically reached solution, and further that we will not have long to wait for a satisfactory explanation of the nonsugar reducing action of blood.

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—*V. C. M.*

The Significance of Achlorhydria in Pernicious Anemia

OFTEN the discovery of the cause of a disease leads to improvement in treatment. New methods of treatment of syphilis followed rapidly after the demonstration of the specific organism of the disease. Occasionally this sequence of events is reversed and the successful treatment of a disease results in new light on etiology as illustrated by new developments in pernicious anemia.

The exact cause of pernicious anemia is not settled. Recent work offers, however, much evidence relative to the etiologic factor or factors concerned in the characteristic clinical symptoms and pathologic picture. The two constant findings in the disease, which together are almost pathognomonic, are the achlorhydria and the macrocytosis of the red blood cells. The macrocytosis is an expression of a difficulty in maturation or normal growth of the erythrocytes. The exact meaning of the achlorhydria has not been clear although every student of the disease considers the absence of free hydrochloric acid in the stomach as a clinical finding necessary for the diagnosis of the disease. When pernicious anemia was considered a hemolytic disease, it

seemed probable that the development of a toxin responsible for the blood destruction might be facilitated by the absence of free acid in gastric digestion. Certainly the hemolytic conception of the disease and probably all toxin theories of the disease must be given up. The primary trouble in blood regeneration is probably the lack of some substance which normally influences the bone marrow and is necessary for normal red cell formation.

The demonstration by Minot and Murphy[1] of a specific antianemic factor in liver has not solved the problem of the actual cause of the disease, although offering convincing evidence that the disease is a deficiency one. It is recognized that the principle is not specific to the liver although this organ is still the most satisfactory source of supply. Many other organs contain it. Recently it has been shown by Zerfas and Koehler that adrenal extract may have a stimulating effect similar to liver extract. The primary source of the specific principle is as yet unknown. It is quite possible for instance that the liver is only a storage point and not a site of formation. New developments throw some light on this phase of the question.

Castle[2] in studying the relation of achlorhydria to pernicious anemia found that the stomach of normal persons secreted a substance which could develop from meat the principle necessary for the maturation of red blood cells. Meat when digested with normal gastric juice gave the typical reticulocyte response when fed to patients with pernicious anemia. Beef muscle digested with hydrochloric acid and commercial pepsin gives no such an effect. These results suggest in view of the constancy of the achlorhydria that the principle responsible for the normal maturation of red cells is a gastric secretion other than hydrochloric acid or pepsin. The achlorhydria is only an evidence of this lack of some principle responsible for the disease rather than being itself responsible for the disease.

Later work adds further evidence of the truth of this view. Sturgis and Isaacs[3] and Sharp[4] have found that desiccated stomach of the hog when fed to patients with pernicious anemia produces the blood changes characteristic of liver extract and a remission in the disease. Conner[5] reports similar results. Sturgis and Isaacs conclude that "the observations so far are in accord with the idea that patients with pernicious anemia evidently have lost or have never had the ability to secrete a substance in their stomachs which has the power to produce blood maturing substance from food." Thus the evidence accumulates that pernicious anemia is a deficiency disease. The lack of a necessary principle leads to difficulty in bone marrow function as evidenced by the macrocystosis of the red cells. The evidence further indicates that the deficiency is primarily a gastric one, the disturbance in gastric secretion being shown objectively by the achlorhydria.

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—R. L. H.

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News Item

The Ward Burdick Research Gold Medal presented annually by the American Society of Clinical Pathologists for the outstanding piece of research done by one of its Fellows, was awarded this year to Dr. H. J. Corper, Denver, Colorado, for his work on isolating and culturing the tubercle bacillus. Dr. Corper was also made President-Elect of the American Society of Clinical Pathologists at the recent convention held in Detroit.